

# CANADIAN JOURNAL OF PLANT SCIENCE

---

---

VOLUME 38

---

---

*January, April, July and October*  
1958

EDITED BY THE  
AGRICULTURAL INSTITUTE OF CANADA  
OTTAWA, CANADA

*Published quarterly by the authority of the  
Chairman of the National Committee on Agricultural Services*



# CANADIAN JOURNAL OF PLANT SCIENCE

VOLUME 38

JANUARY 1958

No. 1

## CONTROLLED LOW TEMPERATURE TESTS OF SPROUTED SEEDS AS A MEASURE OF COLD HARDINESS OF WINTER WHEAT VARIETIES<sup>1</sup>

J. E. ANDREWS<sup>2</sup>

*Canada Department of Agriculture, Lethbridge, Alberta*

[Received for publication March 21, 1957]

### ABSTRACT

Twenty winter wheat varieties were tested for cold hardiness in the sprouted seed stage. Their relative cold hardiness at this stage was in close agreement with their relative cold hardiness in freezing tests of young plants and with their field winter hardiness. Testing by the procedure outlined is apparently a reliable method of testing for cold hardiness. It permits the testing of a large number of varieties in a limited space.

### INTRODUCTION

Winter hardiness of winter wheat can be determined in the field only under conditions suitable for differential winterkilling. It is usually necessary either to grow material in tests for many years before a winter of the desired severity occurs, or to plant it in many locations with the hope of getting the desired degree of killing at one or more of the locations. This makes it difficult to test the optimum number of plants from a population and is a serious drawback in breeding for winter hardiness.

The development of adequate laboratory methods of testing for winter hardiness that could supplement or replace field tests has been the object of much investigation. Freezing under controlled conditions showed the greatest promise (1, 6, 10, 11). A controlled freezing test, which gave high positive correlations with field results, was described by Weibel and Quisenberry (10). This test involved growing varieties in flats, hardening them outside for at least one month, determining an appropriate freezing temperature, and freezing in a cold chamber. The hardening of the plants and the freezing temperature necessary to obtain differential mortality were influenced by weather conditions during the hardening period. Maximum hardiness and best agreement with field results were obtained when the freezing tests were made in December. There was a difference between varieties in their seasonal trend of increased hardiness. Others (3, 5, 7, 8, 9, 11) also found that, with field hardening, the degree of resistance to artificial freezing was influenced by day length, temperature, and radiation; and that varieties responded differently to these conditions.

<sup>1</sup>Contribution No. 217, Cereal Crops Division, Experimental Farms Service.

<sup>2</sup>Senior Cerealist, Cereal Breeding Laboratory, Experimental Farm, Lethbridge, Alta.

Ausemus and Bamberg (2) hardened young plants in a cold chamber prior to freezing. They found little relationship between winterkilling in the field and survival in controlled freezing tests with strains of wheat that differed little in winter hardiness.

Laboratory freezing tests available to date have been used to a limited extent by plant breeders in North America. The reason for this is probably a lack of facilities and necessary help to give constant attention to the tests, the variability in results under different hardening conditions, and the large amount of variability unaccounted for, even though significant correlations with field results are obtained.

The experiments reported herein were undertaken to test the assumption that the cold resistance of winter wheat varieties could be determined by low temperature tests of sprouted seeds before emergence of the leaf from the coleoptile. Testing at this stage should avoid the variability caused by varying light intensities and temperatures during any hardening process as the seedling is still dependent on food reserves in the seed rather than on photosynthesis. It also would allow the testing of a larger number of varieties in a limited space than would testing at more advanced stages of growth.

Recently, Grahl (4) has described a method of testing for cold resistance in the coleoptile stage. Seeds were germinated at room temperature until the coleoptiles were 5 mm. long, hardened for 3 days at 0°C., then frozen at -5.7°C. After thawing for one day at 0°C., they were transplanted to the greenhouse where cold resistance was assessed after 14 days.

#### MATERIALS AND METHODS

The varieties used in this study, in addition to the locally grown winter wheat variety Kharkov 22 M.C., were some of those used by Weibel and Quisenberry (10) in their controlled freezing studies. These latter varieties were chosen to enable comparison of these tests with those of the above-mentioned authors and also because of the large amount of information available on their relative winter hardiness under field conditions. All seed used was produced at the same location and in the same year.

Plastic containers,  $3\frac{1}{2} \times 7$  inches, with eight square compartments and a long centre one were used in the tests (Figure 1). For tests with 20 varieties, the long centre compartment was divided in two, so that there were ten compartments in each container. Fifteen cc. of a dry mixture of two parts washed, sieved fine sand and one part fine vermiculite (Zonolite) were placed in each compartment as a substrate for growth.

A nutrient solution was supplied to the test plants after removal from controlled temperature chambers. It was prepared with tap water and its composition was as follows:

Salt	Concentration in millimoles
$\text{KH}_2\text{PO}_4$	1
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	2
$\text{KNO}_3$	5
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	5



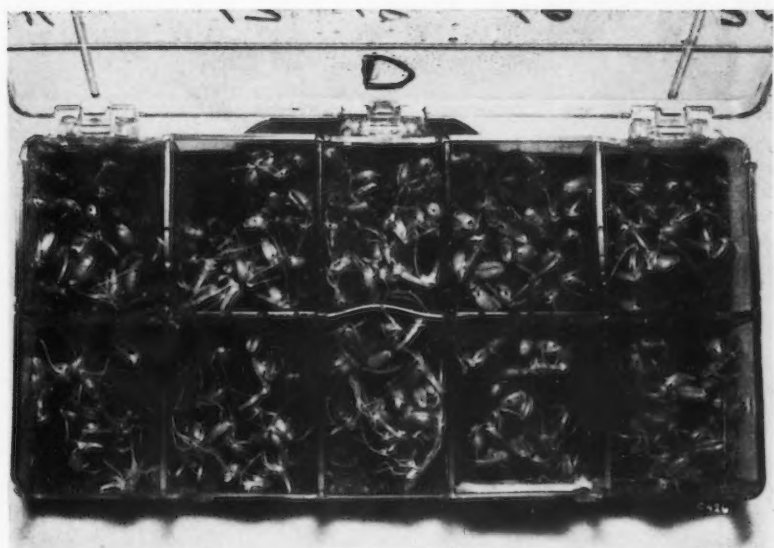


FIGURE 1. Plastic boxes used in freezing tests containing ten winter wheat varieties and showing the stage of development immediately after removal from the freezing chamber.

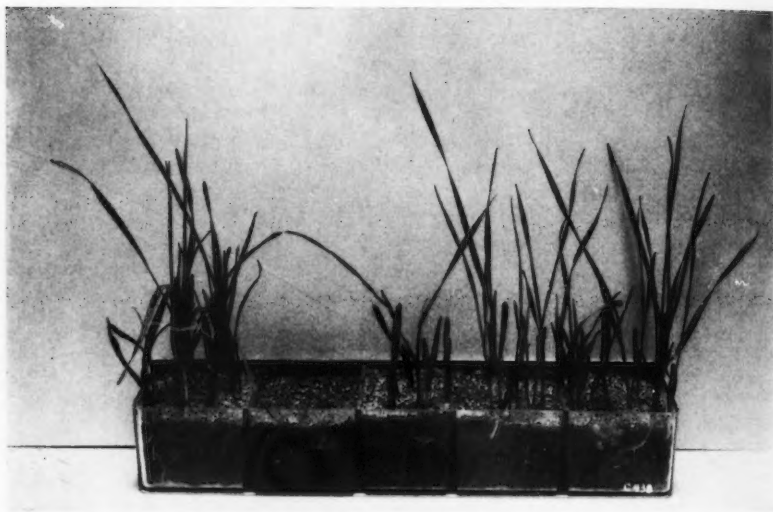


FIGURE 2. Relative survival of five winter wheat varieties, 2 weeks after freezing treatment. From *left to right*—Kharkof C.I. 1442, Blackhull C.I. 6251, Kanred C.I. 5146, Akron Selection C.I. 11660, and Minhardi C.I. 5149.

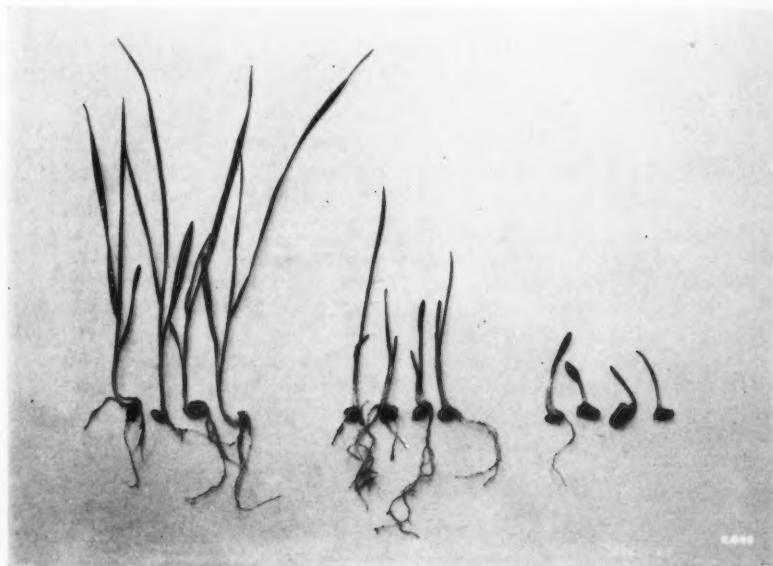


FIGURE 3. Surviving seedlings 2 weeks after freezing showing three levels of damage other than death.

To each litre of this solution was added one ml. of a stock solution of micronutrient elements prepared as follows:

<i>Ingredient</i>	<i>Grams per litre</i>
H <sub>3</sub> BO <sub>3</sub>	2.86
MnSO <sub>4</sub> ·4H <sub>2</sub> O	2.15
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.08
H <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O	0.02

All seed used in the tests was treated with Ceresan to control fungus growth. Damaged seeds were not used. Varieties were placed at random in the compartments of the plastic containers. Twenty-five seeds per compartment were used in one test and 20 seeds per compartment in the other two tests. Ten replicates were used in each of three separate experiments.

With a few exceptions, which are recorded, the testing procedure was similar for all experiments. The seed was placed on the surface of the sand-vermiculite mixture and watered with distilled water. In the first test, sufficient water was added to allow a slight excess on the surface. In the other two tests, 7 ml. of water per compartment were used. After watering, lids of the containers were closed and the material was kept at room temperature (about 22°C.) for 16 hours. It was then moved to a constant temperature room maintained at 0.5°C.  $\pm$  0.5°C. for a period of cold treatment. This period was 4 weeks for the first two tests and 6 weeks for the last test. After the cold treatment, the material was placed, for a period of 16 hours, in a freezing chamber controlled at -15°C. After removal from the freezing chamber, the seed was covered with a sand-vermiculite mixture and placed in a shaded greenhouse maintained at about 20°C. The nutrient solution was added as necessary.

Survival counts were made 2 or 3 weeks after exposure to freezing conditions. All percentage data were transformed by means of the angular transformation ( $\text{Angle} = \arcsin \sqrt{\text{percentage}}$ ) before analysis of variance was applied.

Results of the freezing tests were compared with those from the freezing tests of young plants reported by Weibel and Quisenberry (10), and with field data from uniform winter hardiness nurseries. The field data, summarized by Weibel and Quisenberry (10), gave average plant survivals in percentage of those of Kharkof C. I. 1442 and were based upon 21 to 201 tests conducted during the period 1920 to 1938.

#### GROWTH BEFORE AND AFTER FREEZING

Growth continued very slowly during the cold treatment period at 0.5°C. After 4 weeks in the cold chamber, coleoptiles were 7 to 10 mm. long and roots 20 to 30 mm. long. The extent of growth just after removal from the freezing chamber is shown in Figure 1.

There was considerable variation between and within varieties in their response to the freezing treatment. The condition of the seedlings two weeks after removal from the freezing chamber is illustrated in Figures 2 and 3. The surviving seedlings could be classified into three general

classes (Figure 3). Seedlings in *Class 1* apparently suffered little damage from low temperatures and made rapid growth; those in *Class 2* grew slower than those in *Class 1* and were somewhat stunted. Those in *Class 3* made some initial growth but were severely stunted and usually died within three weeks after the freezing treatment.

The survival index based on the three classes of surviving seedlings was calculated for each variety. Each plant in *Class 1* was given a value of 100, each in *Class 2* a value of 66.6 and each in *Class 3* a value of 33.3. All seedlings that were killed by freezing were given a value of zero. The sum of these values divided by the original number of seeds per compartment gave the survival index. An index value of zero indicated no survival and one of 100 no apparent damage.

#### VARIETAL REACTION IN FREEZING TESTS OF SPROUTED SEEDS

The reaction of winter wheat varieties to controlled low temperatures in the sprouted seed stage is given in Table 1. In *Test 1*, the percentage survival was determined by counting all living seedlings 3 weeks after removal from the freezing chambers. No survival index was calculated.

TABLE 1.—SURVIVAL OF WINTER WHEAT VARIETIES IN CONTROLLED FREEZING TESTS OF SPROUTED SEEDS

Variety <sup>1</sup>	C.I.No.	Angular transformation of				
		Per cent survival			Survival index	
		Test No. 1	Test No. 2	Test No. 3	Test No. 2	Test No. 3
Lutescens 0329	8896	46.3	54.0	31.6	47.4	34.1
Minhardi	5149	42.7	55.2	26.3	44.1	19.4
Minard × Minhardi	8888	46.7	47.4	36.6	38.4	32.3
Minturki	6155	30.6	58.7	32.0	51.2	26.4
Turkey × Buffum	11741	39.6	33.1	16.1	27.3	18.0
Marmin	11502	25.2	56.8	30.3	46.6	25.6
Minturki × Marquis	11659	37.4	47.9	26.3	40.7	22.1
Minturki × Marquis	11501	40.7	42.7	30.0	33.7	24.4
Nebred	10094	27.9	50.1	30.8	41.0	23.7
Turkey × Buffum	11739	39.3	47.7	25.1	38.9	21.8
Cheyenne Selection	11666	37.1	—	—	—	—
Nebraska 60	6250	36.7	50.5	26.3	42.2	21.6
Cheyenne	8885	—	41.9	17.8	32.2	15.8
Kanred	5146	31.3	38.7	11.3	31.8	12.2
Kharkof	1442	22.7	49.6	30.7	43.6	22.0
Akron Selection	11660	32.1	43.5	28.5	36.3	22.0
Blackhull Selection	11737	20.4	24.7	11.1	21.7	8.4
Blackhull	6251	19.3	19.9	7.6	14.6	7.3
Fulcaster	6471	14.2	39.1	17.2	32.6	14.7
Kharkov 22 M.C.	6938	—	57.2	46.5	48.3	37.3
Minhardi × Minturki	8215	—	46.4	43.5	36.1	34.3
Mean		32.8	45.2	26.4	37.4	22.2
Standard Error		4.4	3.8	4.0	2.0	3.0
C.V.		13.4	8.4	15.2	5.3	13.5
L.S.D. (0.05)		12.2	10.6	11.2	5.9	8.3

<sup>1</sup>With the exception of the last two, the varieties are arranged in descending order of field winter hardness



FIGURE 4. Comparison of relative survival of winter wheat varieties in freezing tests of sprouted seeds with that in freezing tests of young plants and in field tests.

In Tests 2 and 3 a survival index rating was made 2 weeks after exposure to freezing and percentage survival calculated 3 weeks after exposure to freezing. There was a significant difference between varieties in their reaction to the low temperature in all tests. In some tests, the standard error in percentage of the test means was quite high, as was the least significant difference between variety means. However, these are not unusually high when compared to field results in years of severe winterkilling. For example, in 1956, when winterkilling was severe in Alberta, the average survivals of 100 plots of Kharkov 22 M.C. winter wheat under each of four different conditions of seeding were 53.4, 62.9, 60.7 and 24.2 per cent with corresponding standard errors of 22.8, 19.6, 31.9 and 29.0 per cent.

#### COMPARISON OF RESULTS FROM CONTROLLED FREEZING TESTS AND FIELD TESTS

The relative performance of varieties in the controlled low temperature tests of sprouted seeds was compared with that in the freezing tests of plants reported by Weibel and Quisenberry (10) and that in the field, by means of correlation coefficients (Table 2) and graphs (Figure 4). Correlations were significant between each of the sprouted seed tests and both the freezing test of plants and the field performance. The higher correlations obtained when data from the three sprouted seed tests were averaged indicated the desirability of a large number of replications to distinguish between varieties that differ only slightly in winter hardiness. The average performance of varieties in the sprouted seed tests was more closely associated with field data ( $r = .85$ ) than with controlled freezing test data of young plants ( $r = .75$ ).

TABLE 2.—CORRELATIONS OF DATA FROM LABORATORY FREEZING TESTS OF SPROUTED SEEDS WITH THOSE FROM FREEZING TESTS OF YOUNG PLANTS AND WITH FIELD SURVIVAL

Sprouted seed tests	Freezing of plants	Field survival
	r	r
Test 1 (Per cent survival)	.82**	.84**
Test 2 (Per cent survival)	.52*	.67**
Test 2 (Survival index)	.56*	.65**
Test 3 (Per cent survival)	.59**	.65**
Test 3 (Survival index)	.84**	.76**
Average 3 tests (Per cent survival)	.75**	.85**

r—correlation coefficients

\*indicates significance at the 0.05 level

\*\*indicates significance at the 0.01 level

The spread in survival between the hardiest and the least hardy varieties (Figure 4) was greater in the low temperature test of sprouted seeds than in the field but not as great as in the freezing test of young plants reported by Weibel and Quisenberry (10). With few exceptions the varieties ranked about the same in relation to the standard check, Kharkof C.I. 1442, under the three methods of testing. The varieties *Lutescens* (C.I. 8896), *Minhardi* (C.I. 5149), *Minard* × *Minhardi* (C.I. 8888), and *Minturki* (C.I. 6155) had the highest survival, while *Blackhull* (C.I. 6251), *Blackhull Selection* (C.I. 11737), and *Fulcaster* (C.I. 6471) had the lowest. *Kharkov 22 M.C.*, which is considered the most winterhardy commercial variety in Canada, had the highest survival in the two low temperature tests in which it was included (Table 1), but comparable data were not available on its reaction in the field or in the controlled freezing test described by Weibel and Quisenberry (10).

Among the exceptions in the ranking of the varieties, *Cheyenne* (C.I. 8885) and *Kanred* (C.I. 5146) gave comparatively lower survivals in both controlled freezing tests than would be expected from their field performance (Figure 4). As these two varieties reacted similarly in the two freezing tests, it is possible that their higher relative survival in the field was the result of factors other than cold resistance. The reaction of C.I. 11741 differed considerably under the three testing methods.

#### DISCUSSION AND CONCLUSIONS

Comparison of the data from the controlled low temperature test of sprouted seeds with field data from uniform trials shows that this low temperature test is a useful method for testing for cold hardiness. The method permits testing of many varieties in a limited space. The number of replicates used will depend upon the degree of distinction required between levels of cold hardiness. Results indicate that the number should not be less than 10. Further refinement of the technique may reduce variation and permit less replication.

It is necessary to keep the average temperature during the cold treatment as near to 0°C. as possible without freezing. A small increase in average temperature results in increased elongation of coleoptiles and consequently abnormal growth when the material is covered after removal from the freezing chamber. It is also necessary to pay special attention to the control of growth of microorganisms.

It should be pointed out that part of the technique was adopted for convenience rather than from necessity. For example, the length of the cold treatment period was selected so that the material would also be vernalized. The effects of a shorter cold period or no cold treatment at all are unknown. A longer cold period is undesirable because of excessive growth. The use of the survival index rather than percentage survival resulted in slightly lower standard errors (Table 1) and slightly higher correlation coefficients (Table 2). However, for large scale tests, it seems unlikely that the slight gain in efficiency would warrant the additional labour of calculating the index.

#### ACKNOWLEDGEMENT

Grateful acknowledgement is made to D. S. Smith, Field Crop Insect Laboratory, Lethbridge, Alta., for supplying the nutrient solution used in the study.

#### REFERENCES

1. Anderson, Arthur., and T. A. Kiesselbach. Studies on the technic of control hardiness tests with winter wheat. *J. Amer. Soc. Agron.* 26:44-50. 1934.
2. Ausemus, E. R., and R. H. Bamberg. Breeding hard red winter wheats for the northern great plains area. *J. Amer. Soc. Agron.* 39:198-206. 1947.
3. Dexter, S. T. Effects of periods of warm weather upon the winter hardened condition of a plant. *Plant Physiol.* 16:181-188. 1941.
4. Grahl, Adolf. Kälteresistenz des Weizens im Koleoptilenstadium. *Landbauforschung, Volkenrode* 6:20-22. 1956.
5. Kneen, Eric, and M. J. Blish. Carbohydrate metabolism and winter hardiness of wheat. *J. Agr. Research* 62:1-26. 1941.
6. Martin, J. H. Comparative studies of winter hardiness in wheat. *J. Agr. Research* 35:493-534. 1927.
7. Suneson, C. A., and George L. Peltier. Cold resistance adjustments of field-hardened winter wheats as determined by artificial freezing. *J. Amer. Soc. Agron.* 26:50-58. 1934.
8. Suneson, C. A., and George L. Peltier. Effect of stage of seedling development upon the cold resistance of winter wheats. *J. Amer. Soc. Agron.* 26:687-692. 1934.
9. Suneson, C. A., and George L. Peltier. Effect of weather variants on field hardening of winter wheat. *J. Amer. Soc. Agron.* 30:769-778. 1938.
10. Weibel, R. O., and K. S. Quisenberry. Field versus controlled freezing as a measure of cold resistance of winter wheat varieties. *J. Amer. Soc. Agron.* 33:336-343. 1941.
11. Worzella, W. W., and G. H. Cutler. Factors affecting cold resistance in winter wheat. *J. Amer. Soc. Agron.* 33:221-230. 1941.



# INHERITANCE OF PERSISTENT SEPALS IN GREEN SPROUTING BROCCOLI<sup>1</sup>

D. R. SAMPSON

*Canada Department of Agriculture, Ottawa, Ontario*

[Received for publication April 24, 1957]

## ABSTRACT

Persistent sepals in broccoli are controlled by *ps*, a single recessive gene. Intermediate phenotypes sometimes occur. *Ps* is linked with the *S* locus.

## INTRODUCTION

During a study of the genetics of self-incompatibility in Calabrese green-sprouting broccoli (2), two plants with persistent sepals were found. The sepals of a broccoli flower usually turn yellow, wither and fall off when the petals drop. In plants with persistent sepals, the petals fall a few days after anthesis as usual, but the sepals remain green, often become somewhat thickened, and stay attached for several weeks (Figure 1). Occasionally one or two sepals persist until the silique matures. Persistent sepals usually become divergent at anthesis, or shortly after, and form a 90° angle with the pedicel, whereas, until they drop, normal sepals remain more or less appressed to the basal part of the petals. After petal-fall, flowers with persistent sepals remind one of tiny propellers.

This study establishes the Mendelian basis for the persistent sepal character, and also demonstrates linkage between the gene for persistent sepals and the *S* locus for self-incompatibility.

## THE GENE FOR PERSISTENT SEPALS

The original plants with persistent sepals were found in a small progeny (4 × 4) which was grown in the greenhouse in 1955. This family was produced by bud selfing plant No. 4 which had normal deciduous sepals. Eight plants of the family also had deciduous sepals, but two (No. 443 and 447) had persistent sepals. This suggested that the persistent sepal character was controlled by a single recessive gene (*ps*). If so, plant 4 would be *Ps ps*, and 443 and 447 would be *ps ps*. To test this hypothesis a much larger population of 4 × 4 was grown in the field in 1956 together with the progeny from 443 × 4 and 443 × 443. The results follow.

### *Family 4 × 4*

This family, which was produced by bud-selfing, was expected to give a ratio of three deciduous to one persistent. No neat segregation into two classes was found (Table 1), because some plants had both types of calyx. These intermediate types were divided into two classes, "mostly persistent" or "mostly deciduous", depending on the degree to which they showed the persistent character. This subjective method of classification doubtlessly resulted in scoring errors.

<sup>1</sup> Contribution No. 898 from the Horticulture Division, Experimental Farms Service.



FIGURE 1. Broccoli racemes showing the two calyx types several days after anthesis.  
*Left*—Persistent sepals; *Right*—Deciduous sepals.

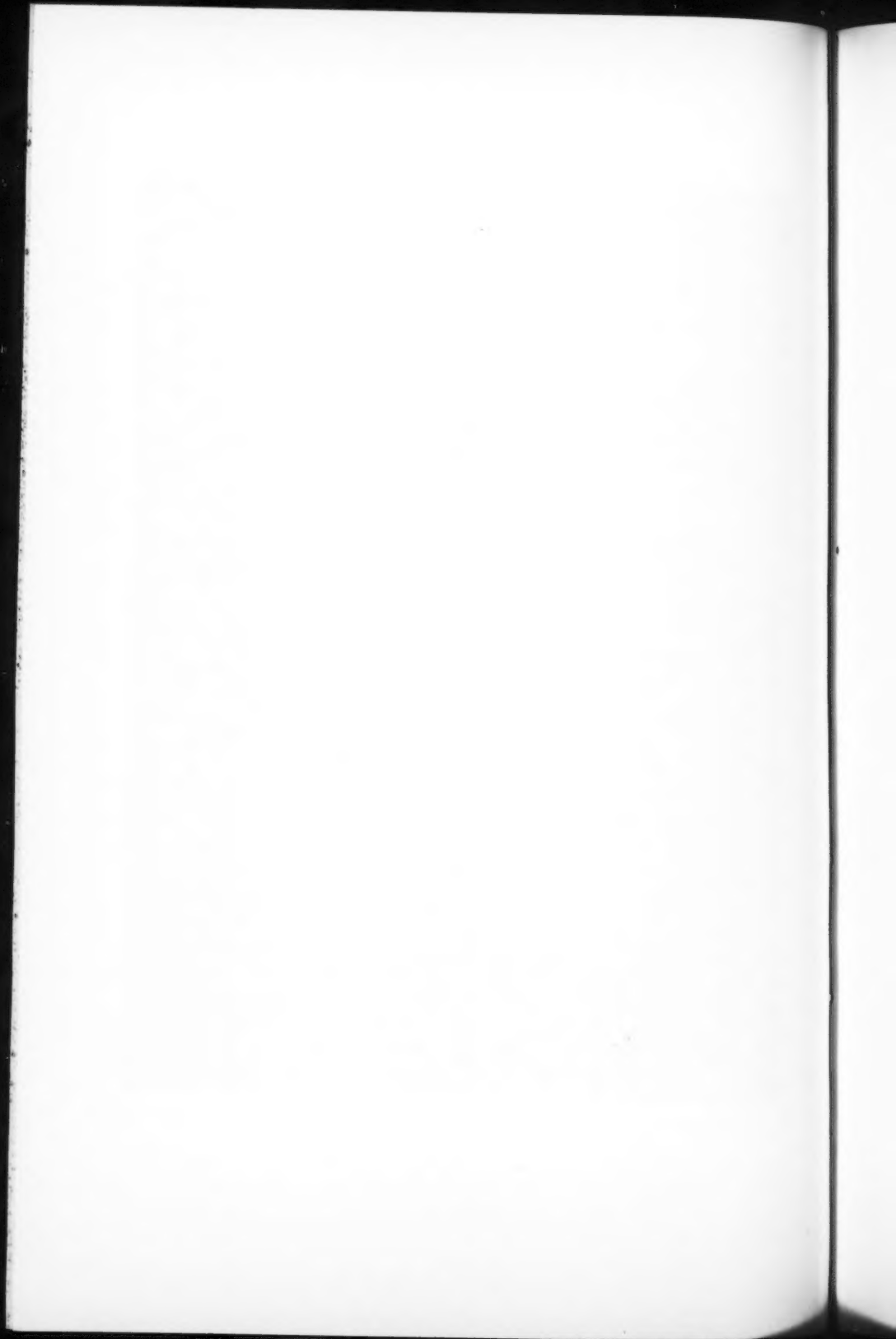


TABLE 1.—SEGREGATION FOR BROCCOLI CALYX TYPES

Cross	Deciduous	Mostly deciduous	Mostly persistent	Persistent
4 × 4 Observed Expected (3:1)	44 64.5	16	8	18 21.5
443 × 4 Observed Expected (1:1)	4 25	20	3	23 25

The presence of intermediate phenotypes agrees with the experience of J. F. Moore\*, Western Washington Experiment Station, Puyallup, Washington. In 1952, Moore discovered a single plant of Calabrese broccoli with persistent sepals. He raised a small progeny in the field but found it difficult to classify. Therefore, the original hypothesis that a completely recessive gene is involved must be altered.

One explanation is that the mostly deciduous class and the deciduous class contain *Ps Ps* and *Ps ps*, whereas the mostly persistent class and the persistent class are *ps ps*. This gives an observed ratio of 60:26, which is close to the expected  $\chi^2 = 1.256$ ;  $.30 > P > .20$ ). However, when both intermediate classes are combined with the deciduous class, the observed ratio of 68:18 is also satisfactory ( $\chi^2 = 0.7597$ ;  $.50 > P > .30$ ).

#### Family 443 × 4

A 1:1 ratio was expected in this family but the occurrence of intermediate classes ruled out this simple explanation (Table 1). A segregation of 24:26 was obtained when each intermediate class was combined with the extreme class it most resembled. A 27:23 ratio was obtained by combining the intermediate and deciduous classes.

#### Family 443 × 443

Since plant 443 supposedly was homozygous recessive (*ps ps*), it should breed true. It was selfed by bud pollination, and the 20 progeny plants all had persistent sepals as expected. The absence of intermediate plants from this family suggests that the intermediates in family 4 × 4 and 443 × 4 were *Ps ps*.

#### LINKAGE BETWEEN *S* AND *Ps*

The foregoing data show that the persistent sepal character is controlled by *ps* which, however, is not completely recessive. Linkage between *ps* and the *S* locus for self-incompatibility was suspected for the following reason: Parent 4 and the original ten plants of 4 × 4 were used in a study of the genetics of self-incompatibility (2). It was found

\* Private correspondence. January, 1957.

that parent 4 had the incompatibility genotype  $S_1S_4$  and that family  $4 \times 4$  segregated for these alleles. However, the only two plants of  $4 \times 4$  with the genotype  $S_4S_4$  (443 and 447) were also the only two plants with persistent sepals, which suggested coupling linkage between  $S_4$  and  $ps$ . Accordingly, parent 4 would be  $S_1Ps/S_4ps$  and 443 would be  $S_4ps/S_4ps$ . Family  $443 \times 4$  provided ideal material for testing this putative linkage.

The linkage study obviously required determination of the  $S$  genotypes in family  $443 \times 4$ .  $S_1S_4$  and  $S_4S_4$  were expected. The determinations were made by applying the pollen of each plant to two female testers, one of which was  $S_1S_3$ , the other  $S_4S_4$ . Since it was known (2) that  $S_4$  is recessive in the pollen of  $S_1S_4$ , this pollen should be compatible on  $S_4S_4$  but incompatible on  $S_1S_3$ . Pollen of  $S_4S_4$ , on the other hand, should be compatible on  $S_1S_3$  but not on  $S_4S_4$ . The use of two contrasting testers ensured against errors caused by inviable pollen. The pollen was brought in from the field and compatibility tests were made in an insect-free greenhouse. Compatibility reactions were determined by the stigma squash method (2). The results and their interpretation follow.

Family  $443 \times 4$ , the product of a cross made at anthesis, segregated into the two expected  $S$  genotypes only. Twenty-nine plants were  $S_1S_4$  and 21 were  $S_4S_4$ . A 1:1 ratio was expected, and the fit is satisfactory ( $\chi^2 = 1.280$ ;  $.30 > P > .20$ ). If there is no linkage between the  $S$  and  $Ps$  loci, each  $S$ -class should contain the four calyx types in the same proportions. Table 2 shows that this was not found. Linkage between  $S_4$  and  $ps$  is therefore clearly indicated. Because of the small population, and the uncertainty of the  $Ps$  genotypes of intermediate plants, the linkage intensity can only be approximated. If the "mostly persistent" class is  $Psps$ , the linkage value is 8 per cent. If the "mostly persistent" class is  $psps$ , the linkage value is 10 per cent. The absence of  $S_4S_4$  plants with deciduous sepals is probably due to chance, because this combination was found in plant 1 and its progeny (2), and in one of the three plants of family  $4 \times 4$ , whose  $S$  genotypes were determined for the present study.

TABLE 2.—OBSERVED DISTRIBUTION OF  $S$  GENOTYPES AMONG THE CALYX-TYPE CLASSES OF FAMILY  $443 \times 4$ , COMPARED WITH THE DISTRIBUTION EXPECTED WITHOUT LINKAGE

	Deciduous	Mostly deciduous	Mostly persistent	Persistent
$S_1S_4$				
Observed	4	20	2	3
Expected	2.32	11.60	1.74	13.34
$S_4S_4$				
Observed	0	0	1	20
Expected	1.68	8.40	1.26	9.66

## CONCLUSIONS

The segregation data for the persistent sepal character can be interpreted on the basis of a single gene and the observed linkage between  $S_4$  and the persistent character is almost conclusive evidence that persistence is due to a single gene. Considering the gene interaction in  $P_s$  heterozygotes, the occurrence of intermediate phenotypes rules out the possibility that  $p_s$  is completely recessive. However, the intermediate classes are too small to include all heterozygotes. Since heterozygotes are not always intermediate, the expression of the gene appears to be variable. Presumably this variability is due to modifying genes or environmental influences.

The genes  $S$  and  $p_s$  bring the number of known genes in green sprouting broccoli to five (1). Some of these mutants can be used as markers to assist in maintaining the purity of varieties. Some can also be used to test the effectiveness of the use of self-incompatibility to produce hybrid seed. However, because of the intermediate classes,  $p_s$  would not be a suitable marker gene.

## REFERENCES

1. Anstey, T. H. Inheritance of white petal in green sprouting broccoli. Can. J. Agr. Sci. 35 : 573-578. 1955.
2. Sampson, D. R. The genetics of self- and cross-incompatibility in *Brassica oleracea*. Genetics 42 : 253-263. 1957.

# YIELD AND CHEMICAL COMPOSITION OF OATS WHEN HARVESTED AT FOUR STAGES OF MATURITY<sup>1</sup>

R. B. MACLAREN<sup>2</sup> AND R. B. CARSON<sup>3</sup>

*Canada Department of Agriculture*

[Received for publication June 10, 1957]

## ABSTRACT

Yield and chemical composition of three varieties of oats harvested at four stages of maturity were studied over a 4-year period. Yields increased as harvesting was delayed from "early dough" to "late dough" and to the "ripe" stage, but decreased when the grain was over-ripe. Percentage of hull decreased with increasing maturity at harvest. There were no significant differences in the proximate constituents due to stage of maturity at harvest. Calcium, phosphorus and magnesium did not appear to be affected by stage of maturity at harvest, but potassium was significantly lower at both the "ripe" and "over-ripe" stages.

## INTRODUCTION

Many farmers in Eastern Canada harvest oats before the crop is fully mature. Since little is known of the effect of such a practice on quality and yield, the present study was undertaken in 1951 and continued through 1954.

Georgeson and others (3, 4, 5) conducted tests in which oats were harvested at three stages of maturity for 3 years but the results were not consistent. Morrow and Gardner (6) obtained their highest yields from plots that were bound and shocked but found no striking effect on the yield or chemical composition of oat grain due to stage of maturity. In his review Arny (1) concluded that the quality of grain was not improved by harvesting crops before they reached full maturity. Arny and Sun (2) found the yield of oats to be variable but indicative of lower yield and bushel weight, and higher hull and protein content when harvested early. This work was continued by Wilson and Raleigh (7) in a year when crown and stem rust were prevalent. They concluded that there was no advantage from premature harvesting of rust-infected oats.

## MATERIALS AND METHODS

Alaska, Abegweit and Victory were used in the project to represent early, medium and late maturing varieties of oats.

Plots were harvested at the early dough, late dough, ripe and over-ripe stages of maturity. At the early dough stage no milk could be forced from kernels taken from the central portion of panicles but glumes, leaves and stems still showed a considerable amount of green colour. Kernels in

<sup>1</sup> Joint Contribution from Cereal Crops Division, Experimental Farms Service, (Contribution No. 219), and Chemistry Division, Science Service, (Contribution No. 362), Canada Department of Agriculture, Ottawa, Ont.

<sup>2</sup> Research Officer, Experimental Farm, Charlottetown, P.E.I.

<sup>3</sup> Head, Analytical Chemistry Unit, Ottawa, Ont.



the late dough stage were reasonably firm but could be dented easily with the thumb-nail. Some colour persisted on glumes and on stems near the nodes. At the ripe stage kernels could be dented only with difficulty and there was little or no green colour on the plants. The over-ripe stage was considered to be 4 or 5 days after the ripe stage.

The crop was cut approximately 4 inches above ground level and bound in sheaves with the heads protected by a cotton sack. After drying for 2 weeks or more the sheaves were threshed, yields recorded and samples drawn for hull percentage, 1000-kernel weight and chemical analysis. In the first year samples for chemical analysis were taken from bulked lots of the four replicates, but in subsequent years samples were drawn from individual plots so that the results could be analysed statistically. In all years hull percentages and 1000-kernel weights for each variety were determined from bulked samples of each stage of maturity of each variety. A split-plot design with four replicates was used with varieties as main plots, and stages of maturity at harvest as sub-plots. Sub-plots consisted of four rows,  $18\frac{1}{2}$  feet long, spaced 9 inches apart. At harvest, the plots were trimmed to one rod in length and the two central rows of each sub-plot were harvested.

Yields were summarized as pounds of groats per acre.

#### RESULTS AND DISCUSSION

Data recorded in 1952 were not used because of seasonal difficulties in harvesting Victory at the four stages of maturity.

A combined analysis of the yields for 3 years indicated significant differences for varieties, years, and stages of maturity at harvest. Significant differences were also recorded for the interactions, stages of maturity by varieties, and stages of maturity by years. However, the variance for stages of maturity was much larger than that for either of the interactions, and it is safe to assume that all varieties are similarly affected by stage of maturity at harvest. For this reason yields and other data are presented as averages for each of the four stages of maturity without regard for variety.

Table 1 indicates that yields at the ripe stage were significantly higher than those at any other stage of maturity. The inferior yield at the over-

TABLE 1.—THREE-YEAR SUMMARY BY STAGES OF MATURITY AT HARVEST

Stage of maturity at harvest	Lb. of groats/acre	% Hull	Gm./1,000 kernels
Early dough	1,739	27.5	30.5
Late dough	1,915	26.3	32.4
Ripe	2,002	25.5	32.5
Over-ripe	1,835	26.0	32.8
L.S.D., P = 0.05	84.2 lb.	1.6%	1.8 gms.

TABLE 2.—THREE-YEAR SUMMARY OF CHEMICAL ANALYSES BY STAGES OF MATURITY AT HARVEST

(Dry matter basis)

Stage of maturity	Protein (Nx6.25) %	Fat %	Crude fibre %	Ash %	N.F.E. %	Calcium %	Phosphorus %	Potassium %	Magnesium %
Early dough	12.6	4.9	11.9	2.8	67.8	0.09	0.41	0.54	0.16
Late dough	12.7	4.8	11.9	2.8	67.8	0.09	0.42	0.55	0.15
Ripe	12.4	4.8	11.5	2.8	68.5	0.10	0.42	0.47	0.15
Over-ripe	12.6	4.7	12.1	2.8	67.8	0.10	0.42	0.41	0.12
L.S.D., P = 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.04	N.S.

ripe stage was, no doubt, due to shattering in the field before harvest, and to loss of kernels at harvest time, caused by the brittle condition of the plants. There was a consistent decrease in hull percentage, from early dough to ripe stage. A slightly higher hull percentage was recorded in the over-ripe plots, but the difference was not large enough to be significant. Weight per 1000 kernels increased progressively from early dough to over-ripe, but the differences obtained in the last three stages were small and insignificant.

Table 2 shows that other than for potassium there were only minor differences in chemical composition due to stage of maturity at harvest. In one year there were significant differences in fat, ash and nitrogen-free extract but when the data for 3 years were combined statistical analysis showed that these differences were not significant. No significant differences were found in protein or crude fibre.

The mineral analyses represent data for 2 years and show that calcium, phosphorus and magnesium were quite uniform at the four stages of maturity but that potassium was significantly lower at the ripe and over-ripe stages.

The experiment shows that for maximum yield, oats should not be harvested until it is mature or nearly mature. Although there were no significant differences in proximate constituents the tendency for slightly lower crude fibre and slightly higher nitrogen-free extract at maturity is indicative of better quality at that stage. Over-maturity should be avoided because of possible loss of crop in the field.

#### ACKNOWLEDGEMENTS

Acknowledgement is made to Miss M. J. MacIntosh, N. H. Goodson and J. H. A. Labelle, who supervised the chemical analyses, and to J. Friesen, who did the statistical work on the chemical data.

## REFERENCES

1. Arny, A. C. The influence of time of cutting on the quality of crops. *J. Amer. Soc. Agron.* 18 : 684-702. 1926.
2. Arny, A. C., and C. P. Sun. Time of cutting wheat and oats in relation to yield and composition. *J. Amer. Soc. Agron.* 19 : 410-439. 1927.
3. Georgeson, C. C., and H. M. Cottrell. Harvesting oats at different stages of ripeness. *Kansas State Agr. Coll. Bull.* 13. 1890.
4. Georgeson, C. C., F. C. Burtis, and William Shelton. Time to harvest oats. *Kansas State Agr. Coll. Bull.* 29. 1891.
5. Georgeson, C. C., F. C. Burtis, and D. H. Otis. Time of harvesting oats. *Kansas State Agr. Coll. Bull.* 54. 1895.
6. Morrow, G. E., and F. D. Gardner. Oats, time and manner of harvesting. Effect upon yield and chemical composition. *Univ. Illinois Bull.* 31. 1894.
7. Wilson, H. K., and S. M. Raleigh. Effect of harvesting wheat and oats at different stages of maturity. *J. Amer. Soc. Agron.* 21 : 1057-1078. 1929.

# WHEAT RUST EPIDEMICS IN WESTERN CANADA IN 1953, 1954 AND 1955<sup>1</sup>

BJORN PETURSON<sup>2</sup>

Canada Department of Agriculture, Winnipeg, Manitoba

[Received for publication February 21, 1957]

## ABSTRACT

Leaf rust and stem rust of wheat (*Puccinia triticina* Erikss. and *P. graminis* Pers. f. sp. *tritici* Erikss. and Henn.) were abundant in the Prairie Provinces of Canada in 1953, 1954 and 1955. Considerable rust damage was caused in all 3 years but particularly in 1954, when both rusts were heavy throughout most of Manitoba and Saskatchewan and in a considerable area in east-central Alberta. In all 3 years, seeding was late and spores arrived early. Abundant moisture favoured rust development throughout the entire seasons of 1953 and 1954 and during the first half of the 1955 season.

In 1953, as a consequence of early arrival of rust spores and an extended period of rainy weather, rust losses were greater than in any year since 1935. Much the greatest damage was caused to durum. In 1954, late seeding, much above normal precipitation over wide areas, and a great northward movement of rust spores at an early date into Saskatchewan, where most of the wheat is grown, resulted in the most severe and widespread rust epidemic in Canada's history. In 1955, high rainfall, late seeding, and a northward movement of rust spores favoured the early establishment of leaf and stem rust, especially in Manitoba. Subsequent rust development was mitigated by two factors: *first*, the onset of hot, dry weather about mid-July, which hastened crop maturity and retarded rust spread; and *second*, the presence of a large acreage in Manitoba and eastern Saskatchewan of the stem- and leaf-rust-resistant variety Selkirk and the leaf-rust-resistant variety Lee, which greatly limited the increase of inoculum of both rusts.

It is estimated that leaf rust and stem rust reduced wheat yields in Western Canada by upwards of 45 million bushels in 1953; 150 million bushels in 1954; and 9 million bushels in 1955.

## INTRODUCTION

Stem-rust-resistant wheats were first introduced into Western Canada in 1936, and by 1939 several stem-rust-resistant varieties (Thatcher, Regent, Renown and Apex) were generally grown in the rust area. From 1939 to 1951, no stem-rust races that could attack these varieties occurred in significant amounts and no stem-rust losses were experienced during these years. A significant increase in race 15B of stem rust, which can attack these varieties, occurred in 1952. Although this race was not present in sufficient intensity to cause appreciable stem-rust losses that year, it was generally distributed on wheat throughout the rust area in Manitoba and eastern Saskatchewan. Increases in the prevalence of race 15B took place during the next 3 years and it occurred in epidemic proportions in 1953, 1954, and 1955 and during this period caused very severe crop losses particularly in 1954 when a very heavy infection spread across Manitoba and Saskatchewan and into eastern Alberta.

It is very difficult to determine rust losses accurately, especially in large areas such as the rust area of Western Canada, which comprises several million acres. The amount of rust usually varies greatly from field to field and from district to district, and heavy-rust areas often merge very gradually into light or non-rusted ones, making average estimates of

<sup>1</sup> Contribution No. 1593 from Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ont.

<sup>2</sup> Plant Pathologist.

rust percentages rather uncertain. Moreover, rust averages used for loss estimates must be taken shortly before harvest and these figures are difficult to obtain over large areas in the short time available to get these data. Furthermore, the amount of damage that a certain percentage of rust may cause varies considerably. Plants which become heavily rusted at an early stage of development and are subjected to rust attack through much of their growing period usually produce little or no yield, while only slight damage may occur if the rust attack, through severe, occurs shortly before harvest.

In experiments carried out at Winnipeg from 1925 to 1932, Greaney (3) showed that in any one year there was a relationship between the amount of stem rust on Marquis wheat and the reduction in yield caused by rust; that is, a given amount of rust caused a definite reduction in yield. In one year—1925—there was a reduction of 9.7 per cent of the possible yield for every 10 per cent of rust; in another year—1932—there was a reduction of 3.1 per cent of the possible yield for every 10 per cent of rust; and for the period 1925 to 1932, the average reduction in yield amounted to 5.4 per cent of the possible yield for every 10 per cent of rust. From Greaney's results it will be seen that rust-loss estimates based on average rust percentages, even when exact rust percentages are available, could be subject to considerable error.

Since rust-loss estimates based on observations of amounts of rust present in the field are subject to several variables it seemed preferable, in the present study, to base the estimate of rust losses on the differences in yield between the highly resistant variety Selkirk and the predominant susceptible varieties. Fortunately, a significant number of comparisons in yield covering much of the rust area were available. It is believed that these yield comparisons give a fairly true estimate of actual rust losses because the fields compared represented not only a large area but also various dates of seeding, and they constituted a random sample of the fields in the rust area.

Attempts were made to determine the losses caused by stem rust in each of the years 1953, 1954, and 1955 as well as the losses caused by leaf rust which also was prevalent and widespread during the 3-year period mentioned.

No attempt was made to assess separately the loss caused by each of the two rusts present. The loss estimate was made by comparing, in the rust area, the yields of the predominant susceptible varieties, Thatcher, Redman and Lee, with the yield of Selkirk wheat which remained almost free of stem rust, carried only very light leaf rust infections and was not anywhere appreciably damaged by rust. Yield comparisons were always made between Selkirk and one or the other of Redman, Thatcher or Lee in farmers' fields where a susceptible variety and Selkirk were grown under comparable conditions.

#### THE 1953 RUST EPIDEMIC

##### *Factors Affecting Incidence of Rusts in 1953*

Owing to dry weather, conditions were not particularly favourable for rust development in Texas and adjoining states during the winter and

spring of 1953. Nevertheless, stem rust made its appearance early in the crop season in the spring-wheat section of the Great Central Plains area of the United States, where, because of high moisture and lush plant growth, conditions were very favourable for rust establishment. Stem-rust infection had become general on wheat throughout much of this region by mid-June. Leaf rust of wheat also became prevalent throughout the same area where stem rust occurred, but leaf rust was somewhat later in becoming well established.

A few spores of both leaf and stem rust were caught on vaselined spore-trap slides during the period May 25 to 28 at Winnipeg, Morden and Brandon in Manitoba, and at Regina in Saskatchewan. But rust spores did not begin to appear in appreciable numbers on the slides exposed in Western Canada until about June 18. From then until the end of the crop season rust inoculum was abundant in the air over Manitoba and eastern Saskatchewan. Throughout the season stem-rust spores were more prevalent in the air than leaf-rust spores, a condition that had not occurred there since 1938, when varieties carrying the type of stem-rust resistance possessed by Thatcher, Renown, Regent, and Redman became the predominant ones grown. Not since 1935 had there been so much total rust inoculum in the air over this area. On a number of occasions, several thousand spores of stem rust and of leaf rust were caught on one square inch of slide during a 72-hour exposure.

Weather conditions during most of the crop season of 1953 favoured rust development throughout the rust area of Western Canada. About one-half of the crop was sown late owing to heavy spring rains; precipitation was much above normal for June and July; and temperatures were near normal for June and July and 4°F. above normal for August. These weather features were very conducive to the increase and spread of rust. However, fairly dry weather prevailed in the western two-thirds of Saskatchewan and Alberta and rusts were not prevalent in these western areas.

#### *Prevalence of Rust in the Field in 1953*

In 1953, wheat-stem rust made its earliest appearance in Western Canada since continuous records were first kept in 1925. A few pustules of this rust were found on Regent wheat at Morden, Manitoba, on June 16. This was 2 weeks earlier than in 1952. The infection spread gradually and by mid-July stem rust was present on wheat in appreciable amounts throughout the agricultural area of Manitoba and eastern Saskatchewan; and before the end of the growing season wheat-stem rust infection ranging from "trace" to "light" was present in western Saskatchewan and Alberta. The westerly and northerly limits of the area of Western Canada severely affected by stem rust in 1953 were roughly definable by a line from the International Boundary directly south of Weyburn, northwest to Belle Plaine (east of Moose Jaw) and thence northeastward to Sturgis, and from there directly east across eastern Saskatchewan and Manitoba (Figure 1). Within this area, the average severity of stem rust on Thatcher, Redman and Lee ranged from 5 to 30 per cent, and averaged about 15 per cent in early-sown fields; in late-sown fields, it ranged from 40 to well upwards



of 60 per cent, and averaged about 50 per cent. Approximately half of the wheat acreage in Manitoba and eastern Saskatchewan was sown during a dry period that prevailed throughout the area from April 17 to May 8. Then, owing to heavy rains, very little was sown for a 2-week period. The remainder of the wheat acreage was not sown until late May and early June. Stem-rust infection, on comparable stands, within the region referred to, was fairly uniform with a slight decrease in rust intensity in the northern parts. The amount of rust present in any field in the heavy-rust area was correlated with its date of seeding rather than with its geographical position within this area. To the west of the above-mentioned line rust diminished rather rapidly and the infection ranged from "trace" to "light" in the western half of Saskatchewan. North of the east-west line through Sturgis, in eastern Saskatchewan and Manitoba, stem rust diminished gradually, and it was present in trace amounts throughout much of Alberta, but not in sufficient intensity to cause appreciable damage. As far as could be observed, there was about an equal amount of stem rust in comparable stands of Thatcher, Redman and Lee. All appeared to be about equally susceptible. In the heavy-rust area only slight amounts of stem rust occurred on Selkirk.

Leaf rust of wheat appeared a few days later than usual. The first traces of leaf-rust infection were found on June 23 at Morden in Manitoba. During the early part of the season it was somewhat less prevalent than usual and infection throughout the season was fairly light, averaging about 12 per cent in the early sown crop. However, it became quite heavy on late crops of Thatcher and Redman throughout Manitoba and eastern Saskatchewan. On these late crops leaf rust averaged about 65 per cent. Infection ranged from 5 to 15 per cent in late fields of Lee wheat, and averaged less than 5 per cent in late fields of Selkirk in the heavy-rust area. The heavy leaf-rust area was approximately co-extensive with the heavy stem-rust area. Leaf rust was present throughout the western half of Saskatchewan but infection was generally light. A very light infection of this rust, ranging from "trace" to "slight", occurred over most of the agricultural districts of Alberta.

It was estimated that the average stem-rust and leaf-rust infection in the heavy-rust area amounted to about 30 per cent, for each rust.

The durum wheat varieties, Carleton, Stewart and Mindum carried higher stem-rust infections (up to 80 per cent) particularly in late fields, than the bread wheat varieties. However, in Manitoba some very early-sown durum fields were only lightly rusted (10-30 per cent).

#### *Damage Caused by Rust in 1953*

In this year, yield losses from rust infection were greater in Manitoba and eastern Saskatchewan than in any year since 1935. The wheat acreage in the heavy-rust area (Figure 1) amounted to slightly more than 5,000,000 acres (2,200,000 in Manitoba and about 3,000,000 in Saskatchewan).

Comparable yield returns were received from 57 farmers, scattered throughout the rust area, who grew Selkirk and one of the susceptible varieties Thatcher or Redman on comparable fields. The average yield of Selkirk obtained by these growers amounted to 36.3 bushels per acre while



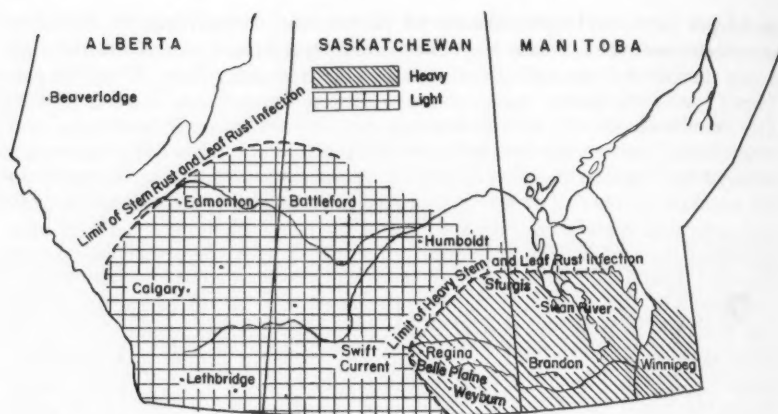


FIGURE 1. Outline map of Prairie Provinces showing areas principally affected by stem rust and leaf rust of wheat in 1953.

the average yield of the susceptible varieties was 25.3 per cent less, or 27.1 bushels per acre. Since the productive capacity of Selkirk and the susceptible varieties, in the absence of rust, is about the same, it is seen that they yielded only about 75 per cent of the amount which would have been obtained had rust been absent. Based on the yield figures given by the Canadian Bureau of Statistics for 1953, it is estimated that the total wheat yield for the heavy-rust area was about 120,000,000 bushels, indicating a possible yield of some 160,000,000 bushels had rust been absent. The loss, therefore, amounted to about 40 million bushels for the heavy-rust area. There were also some considerable rust losses on the western fringe of this area (Figure 1) and in the western parts of Saskatchewan where rust was present but very light. As only a few fields of rust-resistant wheats were situated west of the rust area, no direct method was available for estimating losses there. Rust losses in the western areas were relatively light. However, judged by the amount of rust present, they amounted to about 5 million bushels. The total rust losses, then, for Western Canada amounted to slightly more than 45 million bushels.

#### THE 1954 RUST EPIDEMIC

##### *Factors Affecting Incidence of Rusts in 1954*

In 1954, both leaf rust and stem rust of wheat occurred in epidemic form throughout much of the spring-wheat area of Canada and the United States.

Rusts overwintered in the red stage in northern Mexico and southern Texas during the winter of 1953-54, and by June 1 leaf and stem rust of wheat had become quite prevalent in northern Texas, Kansas, and southern Nebraska. This area then became the source from which rust spores spread northwards across the spring-wheat area.

During the first week in June conditions were favourable for the northward spread of rust. Both leaf- and stem-rust spores in large numbers

were caught on spore-trap slides exposed at Regina, Saskatchewan, and it seems probable that rust spores were carried across Saskatchewan and into Alberta at that time.

The spring of 1954 was moist and cold and seeding was delayed from 2 to 3 weeks in most areas in the Prairie Provinces. This delayed seeding was one of the major causes contributing to the 1954 rust epidemic. In Saskatchewan, precipitation was much above normal. Temperatures were about 1°F. below normal in most prairie areas during the growing season. Temperatures, although slightly below normal, were high enough for rapid rust development. This was demonstrated in rust culture experiments carried out at the Plant Pathology Laboratory, Winnipeg. These experiments showed that the incubation periods for stem rust varied from 7 to 9 days during June, July and August.

#### *Prevalence of Rust in the Field in 1954*

In 1954, stem rust of wheat was first observed at Morden, Manitoba, on June 17, a day later than in 1953. That same week, stem rust of wheat was found throughout the area extending northwestwards from southeastern Manitoba to beyond North Battleford in northwestern Saskatchewan, a distance of over 700 miles. The heavier infections occurred in central and northwestern Saskatchewan, where on June 26, in some fields, traces of stem rust were present on 40 per cent of the plants and traces of leaf rust on 80 per cent of the plants. Usually stem and leaf rust first appear in southeastern Manitoba about mid- or late June and then spread gradually northwestwards, reaching northwestern Saskatchewan 2-3 weeks later. This year, on the contrary, the rusts appeared over this whole area almost simultaneously.

Stem rust became heavy on Thatcher, Redman, and Lee (averaging from 30 per cent to upwards of 70 per cent) in most of Manitoba and Saskatchewan, except southwestern Saskatchewan where rust was light and the northeastern areas where infection was moderate. Durum wheats, in the rust area, carried a much heavier stem-rust infection than bread wheats. Selkirk was very slightly infected. Race 15B comprised over 90 per cent of the wheat stem rust in 1954.

Leaf rust appeared in Manitoba a day earlier than stem rust. It was found in most areas a few days ahead of stem rust and spread somewhat farther north and west. It was very heavy throughout Manitoba and in much of Saskatchewan. Infections averaged from 60 per cent to upwards of 80 per cent on Redman and Thatcher, with the heavier infections on Thatcher. Lee was only slightly affected by leaf rust while light to moderate infections occurred on Selkirk. Redman and Thatcher were defoliated by leaf rust from 2 to 3 weeks before maturity and they probably suffered as much damage from this rust as from stem rust.

#### *Damage Caused by Rust in Western Canada in 1954*

In 1954, stem rust and leaf rust of wheat caused greater losses in Western Canada than in any previous year. An estimated reduction of about 215 million bushels in the expected yield was probably due more to rust than to adverse weather conditions.

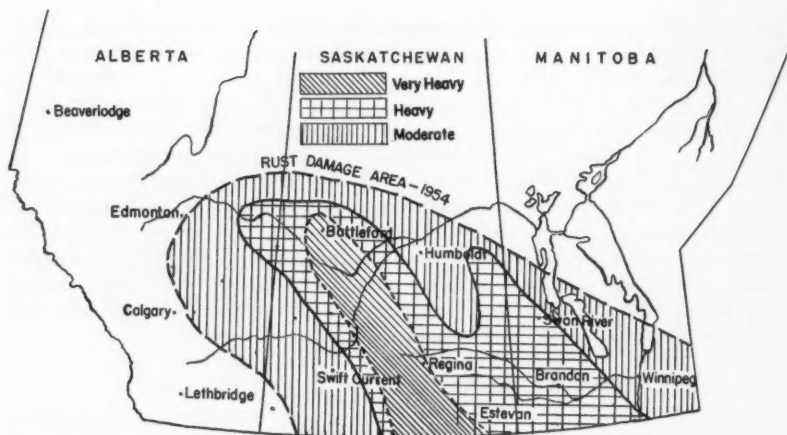


FIGURE 2. Outline map of Prairie Provinces showing areas principally affected by stem rust and leaf rust of wheat in 1954.

Based on the condition of crops on August 1, the Dominion Bureau of Statistics estimated a total wheat production of 487,000,000 bushels of wheat (average of 20.8 bushels per acre) for the Prairie Provinces. Several crop-reporting agencies made similar crop estimates at this time. During the remainder of the season several adverse crop factors—rust, excessive moisture, frost, and hail—caused unprecedented yield reductions. The actual yield amounted to only 272 million bushels, an average yield of 11.6 bushels per acre for the three Prairie Provinces. The greatest yield reduction occurred in central Saskatchewan where yields for some large areas averaged less than 7 bushels per acre. In Alberta, where wheat rusts were least prevalent, the actual yields were closest to the expected yields.

TABLE 1.—COMPARATIVE YIELDS OF SELKIRK, REDMAN, THATCHER AND LEE WHEAT IN 10 CROP DISTRICTS\* IN MANITOBA IN 1954

Crop district	Average yield in bushels per acre			
	Selkirk	Redman	Thatcher	Lee
1	22 (4)	14 (2)	10 (1)	9 (1)
2	37 (22)	16 (6)	11 (13)	18 (3)
3	35 (25)	16 (10)	13 (6)	20 (9)
4 + 5	35 (8)	20 (3)	15 (3)	25 (2)
7	25 (11)	16 (2)	12 (6)	19 (4)
8	35 (16)	17 (6)	10 (7)	33 (3)
9	32 (15)	19 (6)	11 (9)	—
10	40 (40)	20 (6)	17 (34)	—
11	36 (19)	17 (2)	15 (18)	—
13	45 (5)	— (1)	15 (5)	—
Average for Province	35 (165)	17 (44)	14 (102)	21 (22)

Figures in brackets give number of fields

\*The crop districts are those used by the Manitoba Department of Agriculture

TABLE 2.—COMPARATIVE YIELDS OF SELKIRK AND THATCHER WHEAT IN 6 CROP DISTRICTS\* IN SASKATCHEWAN IN 1954

Crop district	Yield in bushels per acre	
	Selkirk	Thatcher
1	33 (25)	11 (25)
2	32 (14)	11 (14)
3	28 (16)	6 (16)
5	34 (73)	12 (73)
6	27 (17)	12 (17)
8	35 (24)	17 (24)
Average for Province	31 (169)	13 (169)

Figures in brackets give number of fields

\*The crop districts are those used by the Saskatchewan Department of Agriculture

An attempt to measure the effect of rust on yield in 1954 was made by comparing the yields of Selkirk, a highly resistant variety, with the yields of the prevalent susceptible varieties. Information on the relative yields of Selkirk and the susceptible varieties was obtained through a questionnaire sent to registered seed growers and contract seed growers by the Cereal Breeding Laboratory, Winnipeg. Information about yields was also obtained through the kindness of the United Grain Growers, Ltd., who made available the results of a questionnaire concerning yields sent to their elevator agents in the rust area.

In 1954, all comparisons were made between pairs of fields of Selkirk and a susceptible variety, which in over 90 per cent of the cases was Thatcher or Redman. Most of the crops compared were grown on summer-fallowed land and were seeded at about the same time. Of the several hundred reports received only the results of those farmers who grew Selkirk and a susceptible variety under strictly comparable conditions were compared.

The questionnaires were sent only to farmers in the heavy-rust area. This area comprised some 11 million acres (Figure 2), 2 million acres in Manitoba, 8 million acres in Saskatchewan, and about 1 million acres in Alberta.

In Manitoba, the average yields (Table 1) were: Selkirk 35 bu.; Lee 21 bu.; Redman 17 bu.; and Thatcher 14 bu., while those for Saskatchewan (Table 2) were: Selkirk 31 bu., and Thatcher 13 bu.

The average yield reduction of the three predominant varieties, Redman, Thatcher and Lee, amounted to about 50 per cent in the heavy-rust area in Manitoba and since the total wheat yield for this area was about 20 million bushels, the estimated yield loss totalled about an equal amount, namely, 20 million bushels.

In the heavy rust area of Saskatchewan and Alberta, extending north-westwards across the former Province from its southeastern corner to beyond North Battleford and for some distance into Alberta, the average yield of wheat amounted to about 7 bushels per acre, or a total yield of about 72 million bushels. The 169 farmers contacted in this region re-

ported a yield of 31 bushels per acre for Selkirk and a yield of 13 bushels per acre for Thatcher, a yield reduction of some 58 per cent for the latter variety. Assuming that the yield difference between Thatcher and Selkirk in the heavy-rust area of Saskatchewan and Alberta was the result of rust infection, the actual rust loss amounted to about 110 million bushels.

The yield reduction then, in the heavy-rust area of Manitoba, Saskatchewan and Alberta (Figure 2) based on a comparison between yields of Selkirk and the predominant susceptible varieties, amounted to about 130 million bushels.

Outside this area to the northeast and to the west a considerable acreage carried a moderate to light rust infection and suffered considerable rust damage which could not be accurately determined owing to the absence of any rust-resistant varieties there to compare with susceptible varieties. However, the amount of rust present would indicate a rust loss of perhaps 20 million bushels.

The total rust loss for the part of the Prairie Provinces inside the area of rust damage (Figure 2) amounted to at least 150 million bushels.

#### THE 1955 RUST EPIDEMIC

##### *Factors Affecting Incidence of Rusts in 1955*

Although conditions were only moderately favourable for overwintering and development of rusts in Texas during the winter of 1954-55, sufficient amounts of rust had developed in northern Texas and in parts of Oklahoma and Kansas to make possible a very considerable northward movement of rust spores at the end of May. Persistent south winds which blew from Texas, May 30 to June 1, deposited spores chiefly over the Dakotas, Manitoba, and to a lesser extent over eastern Saskatchewan.

These spore showers, which contained both stem and leaf rust but chiefly the latter, initiated stem- and leaf-rust infections in southeastern Manitoba several days earlier than usual. Temperatures throughout Western Canada were very favourable for rust establishment and development.

The large acreage sown to the stem rust-resistant variety Selkirk made the establishment of rusts, particularly stem rust, more difficult than in the previous two years. In Manitoba, where the heaviest initial spore showers occurred, and where the first field infections were found, approximately 65 per cent of the wheat acreage (1,250,000 acres) consisted of Selkirk, and there were 1,880,000 acres of this variety in the rust area of Saskatchewan. Stem rust failed to become established on Selkirk, except in trace amounts, and no significant amounts of stem-rust inoculum were produced on it. To a lesser extent Selkirk affected the spread of leaf rust, for this rust increased very slowly on it, particularly during the early part of the season. Lee, a highly leaf-rust-resistant variety, also adversely affected the spread of leaf rust. It was grown on 16.8 per cent and 7.2 per cent of the wheat acreage of Manitoba and Saskatchewan, respectively.

##### *Prevalence of Rust in the Field in 1955*

Stem rust of wheat appeared in Manitoba on June 13, four days earlier than in 1954. It spread across Manitoba and Saskatchewan and

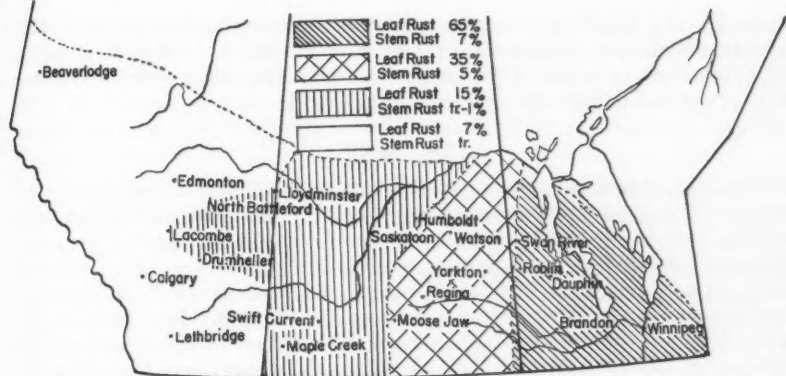


FIGURE 3. Outline map of Prairie Provinces showing areas principally affected by stem rust and leaf rust of wheat in 1955.

into Alberta and varied from light to trace. Infection averaged about 7 per cent in Manitoba and 5 per cent in eastern Saskatchewan, but the rust was present farther west in trace amounts only. The rust percentages mentioned are given in Figure 3.

Leaf rust was first found in Manitoba on June 13. It was co-extensive with stem rust but infection was much heavier, especially in parts of Manitoba. On susceptible varieties infection averaged about 65 per cent in Manitoba and 35 per cent in eastern Saskatchewan, but was much lighter in western Saskatchewan and Alberta. Leaf-rust infection averaged about 15 per cent on Selkirk in Manitoba but in Saskatchewan infection was much lighter on this variety. Variable infection occurred on Lee, but in general was lighter than on Selkirk.

#### *Damage Caused by Rusts in 1955*

As in the two previous years, the estimate of damage caused by leaf and stem rust in Western Canada in 1955 was made by comparing yields of Redman, Thatcher and Lee with those of Selkirk wheat, which remained almost free of stem rust, carried only a very light leaf-rust infection, and sustained negligible rust damage. Yield information was obtained through the United Grain Growers from farmers who, in 1955, grew Selkirk and one or more of the varieties Thatcher, Redman and Lee on comparable land, and sown about the same date.

In Manitoba, where Thatcher and Redman carried light stem-rust infections and heavy leaf-rust infections, these varieties yielded 15.3 per cent less than Selkirk; in the eastern half of Saskatchewan, where both stem and leaf rusts were considerably less prevalent than in Manitoba, Thatcher yielded 7.7 per cent less than Selkirk; and in western Saskatchewan, where stem rust occurred in trace amounts only, and where leaf rust was quite light, Thatcher yielded 1.3 per cent less than Selkirk. Lee yielded 6.2 per cent and 3.6 per cent less than Selkirk in Manitoba and eastern Saskatchewan respectively. Taking into account the acreages of various wheat varieties grown in the three areas mentioned above, and the differences in yield between them and Selkirk, it was estimated that yield



losses due to leaf and stem rust amounted to  $1\frac{1}{2}$  million bushels in Manitoba, 6 million bushels in eastern Saskatchewan, and 2 million bushels in western Saskatchewan, a total of  $9\frac{1}{2}$  million bushels for these two provinces. Wheat did not suffer any appreciable damage in Alberta.

#### DISCUSSION

The development and spread of cereal rusts in Western Canada is governed by a number of different factors and the amount of rust which occurs on cereal crops in this area varies from year to year in accordance with the influence which these various factors exert on both the crop and the rust fungus which attacks it. The main factors which determine the course of rust development are: (1) the amount and time of arrival of the wind-borne rust inoculum which initiates rust each spring in Western Canada; (2) the degree of rust-resistance of the predominant varieties grown to the rust races present; (3) the amount and distribution of precipitation and intensity of dew formation during the crop season (April to August inclusive); (4) the temperature during the crop season; and (5) the time of seeding of the crop.

Craigie (2) studied the relation in Western Canada between the various factors influencing rust increase and the amount of rust present each year for the period 1926 to 1939. No one factor could be singled out as being chiefly responsible for the abundance or scarcity of rust. However, of the several factors studied he found that early arrival of abundant rust inoculum, plentiful moisture, particularly during July, and late seeding were associated with heavy rust years, but that high summer temperatures were less closely associated with severe rust development.

In the Upper Mississippi Valley area, Lambert (4) and Stakman and Lambert (6) showed that there was a close association between rust epidemics and high summer temperatures, particularly July temperatures. Chester (1) found that the amount of leaf rust that develops on wheat in Nebraska is determined by the quantity of spores that survive and increase during late winter rather than by the weather conditions that prevail during the fruiting period of the wheat. Apparently, in that area, weather conditions are usually favourable for spread and increase of rust during the growing season.

The time of arrival of rust spores in appreciable numbers and the distribution of the air-borne inoculum in 1953 had a mitigating influence on subsequent rust development. The fact that spores in appreciable numbers did not appear until about June 21 and that they were concentrated in Manitoba rather than in Saskatchewan, where most of the wheat in Western Canada is grown, tended to minimize the effects of the rust. The distribution of rainfall in Western Canada apparently had a decisive effect on the area covered by the heavy rust infection. The limit of heavy rust infection (Figure 1) coincided almost exactly with the western limit of the area which received precipitation in excess of 4 inches for each of the months of June and July.

Early sowing of about half of the wheat acreage in the rust area allowed that portion of the crop to escape without much rust damage and thus reduced the proportion of the crop which became heavily rusted.



If all this wheat acreage could have been sown early the total rust damage probably would have been quite small. However, the most decisive factor influencing the amount of stem rust present in Western Canada in 1953 was the nature of the rust spores carried into the area. They consisted mostly of race 15B, a race which could attack all the predominant varieties present.

In 1954, the arrival of large numbers of spores of leaf rust and stem rust, June 4-6, almost three weeks earlier than spores in significant numbers came in 1953, and their distribution over much of the wheat crop of Saskatchewan gave rust a good start at an early date and had much to do with severe rust conditions which followed. A very late-sown wheat crop, high precipitation over nearly all of the wheat-growing area throughout the growing season, and the large amounts of race 15B were additional factors which combined to produce one of the most severe wheat-rust epidemics experienced in Western Canada's history.

Temperatures in both 1953 and 1954 were near normal during these two crop seasons. In neither year did they deviate sufficiently to cause any abnormal effect on rust development.

The failure of a severe wheat-rust epidemic to develop in 1955, despite the early arrival of rust inoculum, late seeding and abundant moisture during the early part of the season, can be ascribed to the drought and high temperatures which commenced about mid-July and continued during the remainder of the crop season. The high temperatures hastened the crops to maturity and lack of moisture prevented any appreciable rust establishment after mid-July. Besides drought, another important factor was operative which materially retarded rust increase in 1955: namely, the large acreages (over 3,000,000 acres) of Selkirk wheat on which stem rust occurred in trace amounts only and leaf rust quite sparingly, and (800,000 acres) of Lee wheat, a most uncongenial host for increase of leaf-rust inoculum.

Close observation of the events which led up to the rust conditions which developed in Western Canada in the years 1953 to 1955 demonstrated the difficulty of forecasting rust epidemics any appreciable time in advance. In 1954, for example, the presence of considerable rust inoculum in early June and its distribution over large areas of wheat crops indicated the probability of a rust epidemic. However, even when it was known, about June 23, that both leaf- and stem-rust infections were present throughout all of Manitoba and much of Saskatchewan, it was still impossible to foretell how much rust would develop in July and August without knowing the details of July and August weather in advance. Weather conditions favoured rust and a major epidemic resulted. In Kansas, another part of the Great Plains area, rust conditions almost identical with those existing in Western Canada in late June were present at the beginning of June and, according to Pady and Johnston (5), a major rust epidemic seemed imminent. They stated: "By June 2 it was evident that there would be a major stem-rust epidemic in Kansas unless something unforeseen intervened". Yet an epidemic did not materialize there because exceptionally hot, dry weather, which commenced about June 8,

checked rust increase and hastened the crops to maturity without much further rust development.

In 1955, all the conditions for a major rust epidemic were present in the early part of the season in Western Canada, and rust was prevalent and widespread by mid-July, but a major rust epidemic was averted because of the occurrence of an unforeseen event, namely, a long period of hot dry weather which commenced about mid-July and continued until the end of the crop season.

From observations of the factors influencing the course of rust development during the three years under review, it seems that all these main factors must be present and that at least some of them must be operative throughout the season and must occur over vast areas of the Great Plains region of North America in years when major rust epidemics occur in Western Canada. It would, then, appear that reliable rust forecasts cannot be made for Western Canada until reliable long-time weather forecasting becomes a reality.

#### ACKNOWLEDGEMENTS

Thanks are due to A. B. Masson, Cereal Breeding Laboratory, Winnipeg, Man., who made available the results of questionnaires concerning the yields of Selkirk, Thatcher, Redman and Lee sent to contract seed-growers in 1953 and 1954, and to the United Grain Growers, Ltd., who made available the results of questionnaires sent to their elevator agents on the comparative yields of the above-named varieties in farmers' fields in 1954 and 1955.

#### REFERENCES

1. Chester, K. Starr. The decisive influence of late winter weather on wheat leaf rust epiphytotics. *Plant Disease Reprtr. Suppl.* 143:133-144. 1943.
2. Craigie, J. H. Epidemiology of stem rust in Western Canada. *Sci. Agr.* 25:285-401. 1945.
3. Greaney, F. J. Method of estimating losses from cereal rusts. *Proc. World's Grain Exhibition and Conf.* Volume II:224-236. 1933.
4. Lambert, E. B. The relation of weather to the development of stem rust in the Mississippi Valley. *Phytopathology* 19:1-71. 1929.
5. Pady, S. M., and C. O. Johnston. The concentration of airborne rust spores in relation to epidemiology of wheat rusts in Kansas in 1954. *Plant Disease Reprtr.* 39: 463-466. 1955.
6. Stakman, E. C., and E. B. Lambert. The relation of temperature during the growing season in the spring wheat area of the United States to the occurrence of stem rust epidemics. *Phytopathology* 18:369-374. 1928.

# LONGEVITY STUDIES WITH WHEAT SEED AND CERTAIN SEED-BORNE FUNGI<sup>1</sup>

R. C. RUSSELL<sup>2</sup>

Canada Department of Agriculture, Saskatoon, Saskatchewan

[Received for publication March 5, 1957]

## ABSTRACT

The longevity of several lots of wheat seed with cracked seed coats was compared, over a period of 17 years, with that of several lots of seed with sound coats. At the beginning of the test the average germination of the first group was 4 per cent lower than that of the second. This difference increased to 12 per cent and remained at that level for about 9 years, but for the last 4 years there was little difference in the germination of the two groups. All seed lots germinated well for about 8 years; then the seed lost its viability with increasing rapidity up to the 15th year, after which it deteriorated more slowly until, at the end of 17 years, nearly all the seed was dead.

The longevity of two seed-borne fungi, *Helminthosporium sativum* and *Alternaria tenuis*, on wheat seed was compared. *A. tenuis* disappeared in about 7 years. *H. sativum* lost its viability more slowly. Eight per cent of the original amount was present on the seed at the end of 17 years.

## INTRODUCTION

In 1920, Sifton (3) found that the viability of wheat seed remained unimpaired for 5 years, then fell off with increasing rapidity for the next 10 years, after which it declined less rapidly until it was lost completely by the 19th year.

Recently Machacek and Wallace (1) studied the longevity of wheat, oats, and barley seed and that of certain seed-borne fungi in Manitoba over a 10-year period. They found that after 10 years in storage the average germination of eleven lots of wheat was 66 per cent, but that of individual samples varied from 18 to 85 per cent. In their experiment *Helminthosporium sativum* and *Alternaria tenuis* both lost their viability almost entirely within 10 years and at about the same rate. *H. avenae* on oat seed and *H. teres* on barley retained their viability much longer than did *H. sativum* or *A. tenuis* on wheat.

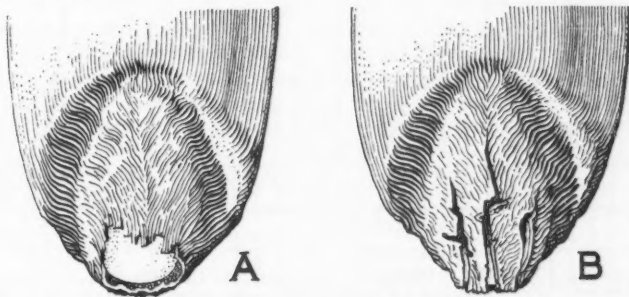


FIGURE 1. Two types of embryo exposure in wheat kernels. (A) A circular area of the seed-coats chipped off, exposing the tip of the coleorhiza, and (B) cracks in the pericarp and testa, where they cover the face of the embryo.

(This illustration appeared in the Reference No. 2, by Mead, Russell and Ledingham.)

<sup>1</sup>Contribution No. 1595 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ont.

<sup>2</sup>Plant Pathologist.

Wheat seed threshed under dry conditions in Saskatchewan frequently shows more or less conspicuous holes and cracks in the seed-coat covering the embryo (2). The object of this experiment was to determine whether or not such damaged seed (Figure 1) would retain its viability as long as seed with unbroken seed-coats. Also it was decided to study the longevity of two seed-borne fungi, *Helminthosporium sativum* and *Alternaria tenuis*.

#### MATERIALS AND METHODS

Most of the wheat seed used in the present study was grown in Saskatchewan in 1939 and 1940. The samples were divided into three groups. The first group, consisting of six samples, carried *H. sativum* and *A. tenuis*.

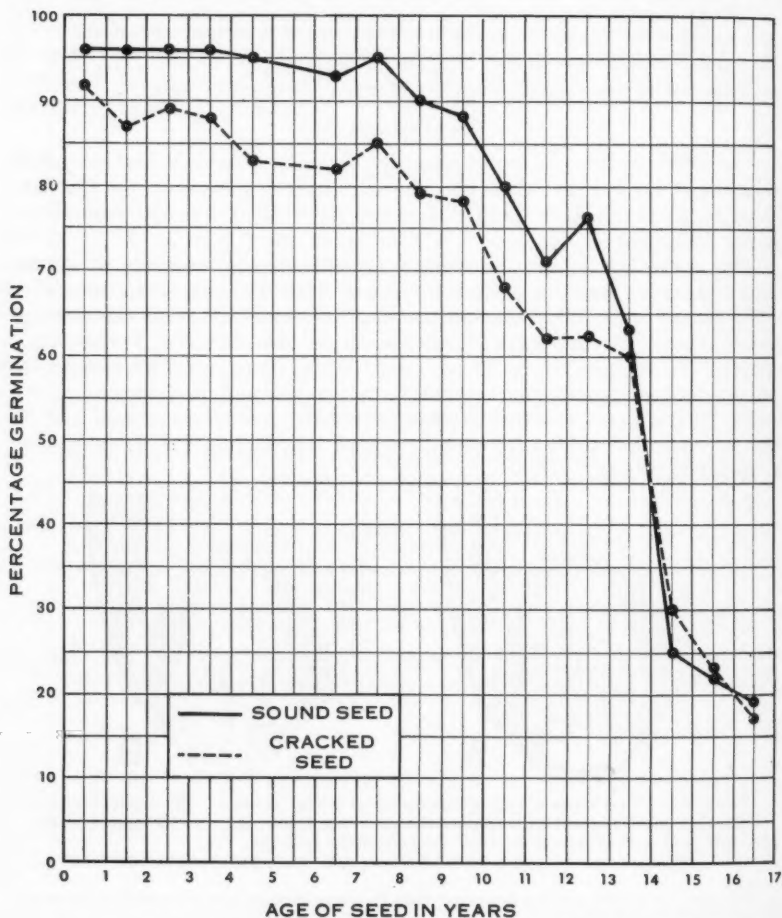


FIGURE 2. Comparison of the percentage germination of wheat seed with cracks in the seed-coats, and that of seed with sound coats, during a period of 17 years.

In this group, from 6 to 9 per cent of the seeds in the Saskatchewan samples carried *H. sativum*; one sample from Alberta carried this fungus on 17 per cent of the seeds, and one from British Columbia carried it on 48 per cent. In the second group, consisting of ten samples, a considerable amount of the seed showed cracks or breaks in the seed-coat over the embryo; on the average, 66 per cent of the seeds were affected. In the third group, which also consisted of ten samples, 92 per cent of the seeds had sound unbroken coats. Varieties represented by the various samples in these groups included Apex, Marquis, Regent, Renown, and Thatcher—all hard, red, spring wheats.

The seed was stored at room temperature in heavy paper envelopes and kept in a tin box which had a loose cover. The seed was not surface-sterilized. Germination tests were made annually on moist filter paper in Petri dishes. Twenty-five seeds were sown in each dish and the dishes were kept in an incubator at a temperature of 24° C. during germination. Either 100 or 300 seeds per sample, depending on the amount of seed available, were sown each time that the tests were made. During the first 12 years portions of the seed were incubated at yearly intervals for 5 days at 72° F.; then the incubation-period was lengthened to 7 days because the aged seed germinated more slowly. At the end of the incubation-period the number of seeds that had germinated was recorded. The percentage of seeds bearing sporulating colonies of *H. sativum* or *A. tenuis* was also recorded.

#### LONGEVITY OF THE WHEAT SEED

The loss of viability over a period of 16 years, shown by the cracked seeds of Group 2 and the sound seeds of Group 3, is illustrated graphically in Figure 2. There was not much loss in viability during the first 7½ years but from then on deterioration was fairly rapid. In the 11th year the seed was kept too wet on the filter paper and the germination of all samples was lower than it should have been. On the average, the germination of the seed in the sound samples was depressed more than it was in the cracked samples, so the difference in the percentage germination of the two groups for that year probably is less than it would have been under more favourable conditions. When the seed was first germinated the average germination of the sound seed was about 4 per cent higher than that of the cracked seed. During the first 4 years this difference in favour of the sound seed increased to 12 per cent and then remained fairly constant for about 8 more years; then the difference disappeared and for the last 4 years the average percentage germination of the two lots was approximately the same. When the seed became aged it was found that it did not germinate as well in soil as on moist filter paper in Petri dishes.

#### LONGEVITY OF *HELMINTHOSPORIUM SATIVUM* COMPARED WITH THAT OF *ALTANARIA TENUIS*

The amount of *H. sativum* and *A. tenuis* appearing in the seed year by year, expressed as a percentage of the infestation found in the first test, is shown in Figure 3. At that time 18 per cent of the seeds carried *H. sativum* and 45 per cent carried *A. tenuis*. Following the second year the amount of *H. sativum* decreased rapidly until the seed was 8½ years old. After fluctuating somewhat for the next 4 years, it dropped to about 8 per cent of the

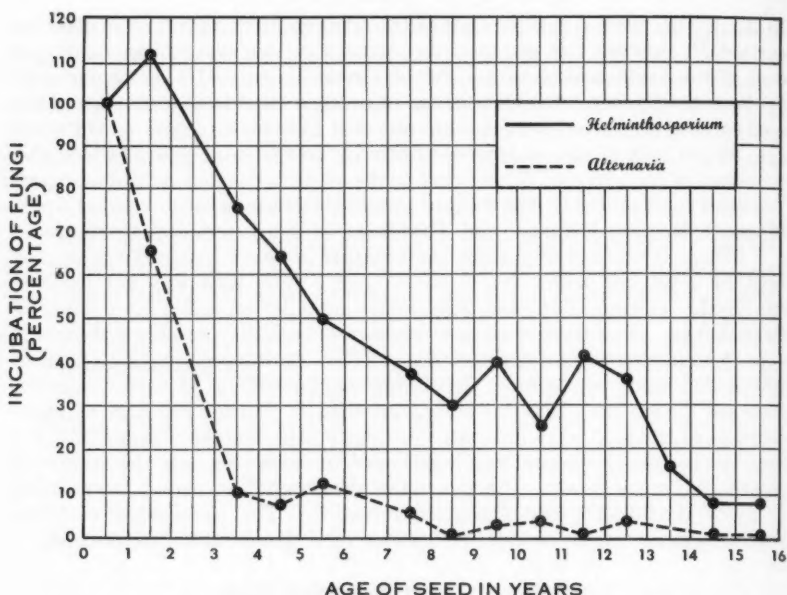


FIGURE 3. Comparison of the longevity of two seed-borne fungi—*Helminthosporium sativum* and *Alternaria tenuis*. The amounts of each are expressed in percentages of the amounts obtained at the initial incubation.

amount that was present originally. Unlike *H. sativum*, *A. tenuis* lost its viability rapidly during the first year. At the end of 3 years it was down to 11 per cent of the original amount. Very little of it appeared on the germinating seed after the 7th year.

#### DISCUSSION

The loss of viability in the wheat seed in this experiment followed closely that of the wheat seed used in Sifton's tests (3). In his tests, about one-half of the seeds died in the 13th year. In the present test, 38 per cent of the sound seed and 30 per cent of the cracked seed died between the ages of  $13\frac{1}{2}$  and  $14\frac{1}{2}$  years. At the end of  $16\frac{1}{2}$  years, in a supplementary test, when the seed was sown to a depth of a half-inch in flats of soil, very few of the embryos produced seedlings that were vigorous enough to emerge from the soil.

The seed in the samples showing considerable seed-coat breakage did not germinate as well as those with sound coats until the final stages of the experiment. The difference between the germination on the two groups was greatest from the 5th to the 13th year and it practically disappeared during the last 4 years.

As for the behaviour of the seed-borne fungi, *H. sativum* and *A. tenuis*, the results were somewhat different from those obtained by Machacek and Wallace (1). In their tests, these two fungi lost their viability at approximately the same rate, while in this investigation *A. tenuis* died out



much more rapidly than did *H. sativum*. *H. sativum* was slightly more abundant in the second year than in the first, but after that it lost its viability fairly rapidly for the next 7 years. However, at the end of 15 years it was still present on a small percentage of the seeds.

The storing of wheat seed for a few years is not a practicable method for freeing the seed of *H. sativum* because the fungus retains its vitality too long.

#### REFERENCES

1. Machacek, J. E., and H. A. H. Wallace. Longevity of some common fungi in cereal seed. *Can. J. Botany* 30:164-169. 1952.
2. Mead, H. W., R. C. Russell, and R. J. Ledingham. The examination of cereal seeds for disease and studies on embryo exposure in wheat. *Sci. Agr.* 23:27-40. 1942.
3. Sifton, H. B. Longevity of the seeds of cereals, clovers, and timothy. *Amer. J. Botany* 7:243-251. 1920.



# CHEMICAL CONTROL OF INSECTS ATTACKING ALFALFA IN SOUTHWESTERN ONTARIO<sup>1</sup>

K. G. DAVEY AND G. F. MANSON<sup>2</sup>

Canada Department of Agriculture, Chatham, Ontario

[Received for publication March 18, 1957]

## ABSTRACT

Surveys in southwestern Ontario in 1953 and 1954 showed that the initial small numbers of sucking insects found in alfalfa fields in early spring tended to increase rapidly as the season progressed. Each time the hay was cut the number of insects was reduced but the invading population soon built up, often to economic proportions. A spray of malathion, perthane, toxaphene, or heptachlor, applied in the spring of 1954, prevented the usual build-up of spittlebug nymphs. Each of the insecticides tested, except heptachlor, increased the yield of the first cutting of hay. An application after each of the first and second cuttings did not give adequate control of sucking insects present and did not increase either cutting of hay.

## INTRODUCTION

Surveys during the summers of 1953 and 1954 near Chatham, Ontario, showed certain species of sucking insects present in alfalfa fields in economically important numbers. Two to three nymphs of the meadow spittlebug, *Philaenus leucophthalmus* (L.), per stem were present during May and June. Adults were plentiful later in the season. The pea aphid, *Acyrtosiphon pisi* (Kltb.), was present in large numbers in some fields. As frequently occurs, the aphid infestation was uneven in those fields infested. The potato leafhopper, *Empoasca fabae* (Harr.), was present from early June until autumn. Various *Liocoris* species, principally *L. lineolaris* (Beauv.) were numerous from May until September.

Insecticidal programs in the United States have controlled these insects so far as seed yields are concerned (3, 4, 8). Reports (5, 7) on programs conducted in forage stands do not include yields. This is a report on an experiment on the effect of chemical control of sucking insects on the yield of hay in a field of alfalfa near Chatham, Ontario.

## MATERIALS AND METHODS

Twenty plots, 120 ft. X 60 ft., in a large field of alfalfa on a privately-owned farm, were treated with insecticides at the following rates per acre: malathion, 0.5 lb.; perthane, 1.0 lb.; toxaphene, 1.25 lb.; heptachlor, 0.38 lb.; and check. Each treatment was replicated four times. The chemicals were applied as wettable powders in 50 gal. of water per acre from a hand-carried boom connected to a John Bean sprayer by 150 ft. of polyethylene garden hose. The sprays were applied at a pressure of 100 lb. per square inch.

Sprays were applied on May 19, when spittlebug nymphs had begun to increase in number, and on July 9 and August 11, when the second and third crops were developing.

<sup>1</sup>Contribution No. 3544, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Ont.

<sup>2</sup>Assistant Entomologist and Entomologist, respectively.

Nymphs of spittlebugs, *P. leucophthalmus*, were estimated by counting the nymphs on 50 stems, selected at random from each plot. Numbers of other insects were estimated by taking 50 sweeps with a 12-in. net in each plot. One count was made before and several after each spray was applied.

Yields were calculated from five random samples from each plot, each from 1 square yard. The alfalfa was cut with garden shears to ground level and weighed immediately. The samples were taken on June 14 and July 20, at the time of normal harvesting operations, and on September 4, when a third cutting would have been made had it not been for dry weather.

### RESULTS

The number of nymphs of the meadow spittlebug present at various dates following the first application of each of the five treatments is shown in Table 1. Each of the treatments reduced the number of nymphs in comparison with the check, in the following order of effectiveness: toxaphene, heptachlor, perthane, and malathion.

Pea aphids were abundant in the experimental plots only until the middle of June. Malathion reduced the number for approximately 10 days, but Table 2 shows that an increase occurred following the application of perthane, toxaphene, and heptachlor. Presumably these chemicals destroyed the parasites and predators without controlling the aphids.

Adults of the meadow spittlebug did not appear in the plots until after the first crop of hay had been harvested. Table 3 shows that none of the insecticides gave satisfactory control of the adults though each of them slightly reduced the number after the second and third applications.

TABLE 1.—NUMBERS OF NYMPHS OF THE MEADOW SPITTLEBUG, *Philaenus leucophthalmus* (L.), PER 200 ALFALFA STEMS SPRAYED MAY 19 AND SAMPLED ON THE DATES SHOWN, CHATHAM, ONT., 1954

Date	Malathion	Perthane	Toxaphene	Heptachlor	Check	L.S.D.*
May 20	3	7	2	7	11	—
May 25	6	1	3	3	83	9.05
June 1	51	19	10	13	235	9.16
June 8	85	39	0	24	527	10.96

\*Least significant difference at the 5 per cent level.

TABLE 2.—NUMBERS OF THE PEA APHID, *Acyrtosiphon pisi* (KLTB.), TAKEN IN 200 NET SWEEPS, 50 SWEEPS IN EACH OF FOUR PLOTS, SPRAYED MAY 19 AND SAMPLED ON THE DATES SHOWN, CHATHAM, ONT., 1954

Date	Malathion	Perthane	Toxaphene	Heptachlor	Check	L.S.D.*
May 20	206	709	1109	1528	792	635
May 25	97	986	1214	1934	1454	660
June 1	343	933	837	1091	962	306
June 8	1399	2686	2274	2975	1871	—

\*Least significant difference at the 5 per cent level.

TABLE 3.—NUMBERS OF ADULTS OF THE MEADOW SPITTLEBUG, *Philaenus leucophthalmus* (L.), TAKEN IN 200 NET SWEEPS, 50 SWEEPS IN EACH OF FOUR PLOTS, CHATHAM, ONT., 1954. SPRAYED ON MAY 19, JULY 7, AND AUGUST 11 AND SAMPLED ON THE DATES SHOWN (THE ALFALFA WAS CUT JULY 20)

Date	Malathion	Perthane	Toxaphene	Heptachlor	Check
July 6	191	273	245	139	202
July 9	148	112	141	129	217
July 13	346	308	213	321	393
July 20	476	628	516	470	608
Aug. 10	20	32	25	16	18
Aug. 13	4	1	7	14	21
Aug. 18	26	20	30	43	39
Aug. 24	206	129	147	189	195
Aug. 30	186	86	124	194	228

Treatments not significantly different.

TABLE 4.—NUMBERS OF THE POTATO LEAFHOPPER, *Empoasca fabae* (HARR.), TAKEN IN 200 NET SWEEPS, 50 IN EACH OF FOUR PLOTS, SPRAYED MAY 19, JULY 7, AND AUGUST 11 AND SAMPLED ON THE DATES SHOWN, CHATHAM, ONT., 1954 (THE ALFALFA WAS CUT FOR HAY JULY 20)

Date	Malathion	Perthane	Toxaphene	Heptachlor	Check	L.S.D.*
July 6	58	52	70	65	43	—
July 9	1	2	49	95	40	32.4
July 13	16	16	29	81	72	28.9
July 20	24	64	148	68	82	36.1
Aug. 10	41	58	42	84	55	—
Aug. 13	0	2	32	43	18	15.0
Aug. 18	17	3	18	51	28	—
Aug. 24	62	31	69	99	65	—
Aug. 30	51	29	48	67	77	—

\*Least significant difference at the 5 per cent level.

The potato leafhopper did not appear until after the first cutting of hay had been removed. The second and third spray applications were, therefore, the only ones which affected this species. Table 4 shows that malathion and perthane gave immediate marked reduction in the number of leafhoppers but the effect had almost disappeared after 2 weeks. Toxaphene was less effective than malathion and perthane. Heptachlor failed to give any reduction in number.

Table 5 indicates that each of the four chemicals reduced the number of *Liocorus* spp. within 2 days of treatment. This is evident following the second, and also the third, spray application, and is most marked in the plots treated with toxaphene and heptachlor. A build-up of numbers in the treated plots is apparent within 1 week of treatment in all cases, and within 2 weeks the effect of all sprays had disappeared.

The average yields of hay are given in Table 6. Student's *t* test showed that each of the treatments, except heptachlor, gave significantly higher yield than the check on the first cutting. Later treatments were less successful, but perthane gave a significant increase in the second cut.

TABLE 5.—NUMBERS OF *Liocoris* spp. TAKEN IN 200 NET SWEEPS, 50 IN EACH OF FOUR PLOTS, SPRAYED MAY 19, JULY 7, AND AUGUST 11 AND SAMPLED ON THE DATES SHOWN, CHATHAM, ONT., 1954 (THE ALFALFA WAS CUT FOR HAY ON JULY 20)

Date	Malathion	Perthane	Toxaphene	Heptachlor	Check	L.S.D.*
July 6	61	79	84	165	66	—
July 9	14	15	6	9	33	12.6
July 13	34	30	38	27	57	13.6
July 20	52	92	160	62	86	50.4
Aug. 10	38	42	35	29	29	—
Aug. 13	1	10	3	4	9	—
Aug. 18	16	19	40	21	20	—
Aug. 24	38	52	59	51	46	—
Aug. 30	30	45	53	33	44	—

\*Least significant difference at the 5 per cent level.

TABLE 6.—YIELDS OF ALFALFA IN OUNCES GREEN WEIGHT PER SQUARE YARD BASED ON THE AVERAGE AVERAGE OF 5 SAMPLES FROM EACH OF FOUR PLOTS IN EACH CUTTING, CHATHAM, ONT., 1954

Date cut	Malathion	Perthane	Toxaphene	Heptachlor	Check	L.S.D.*
June 11	34.9*	40.5*	43.9*	30.0	28.2	6.28
July 20	18.4	24.3	21.3	22.4	19.4	—
Sept. 4	15.6	17.5	16.9	14.8	15.6	—

\*Least significant difference at the 5 per cent level.

## DISCUSSION

This experiment showed that it is possible to increase yields in first cuttings of alfalfa by controlling spittlebug nymphs with malathion, perthane, or toxaphene. Toxaphene increased the yield over the check by 15.7 oz. per square yard, or 4,750 lb. per acre, green weight. According to Albritton (1), the loss in weight from sun-curing hay is about 50 per cent. An increased yield of cured hay of over 1 ton per acre was, therefore, realized in the toxaphene-treated plots. In 1953, the average value of a ton of hay in Kent County was \$15.75 (9). The average cost of treating an acre of alfalfa in 1952 was \$1.42 for the United States (2). The spraying operation was, therefore, economically advantageous.

The failure of heptachlor to increase yields in the first cutting is very puzzling, in view of the fact that it gave excellent control of spittlebug nymphs. Plots treated with heptachlor showed a pea aphid population consistently higher than in the check but not much higher than in the toxaphene-treated areas. Preliminary tests at the Chatham laboratory suggest that heptachlor, under certain conditions which are not clearly understood, may have a phytotoxic effect on alfalfa.

Where temporary reductions in population occurred, as in the case of the leafhoppers, a rapid rise followed the initial decrease. This might have resulted from the destruction of parasites and predators in the treated areas, but more likely because of a rapid re-infestation from the untreated surrounding area.

## ACKNOWLEDGEMENTS

Grateful acknowledgement is extended to Ian Maynard, the grower concerned in the study, to Miss J. I. Evans, Student Assistant at the Chatham laboratory for valuable assistance, and to R. J. McClanahan, also of the Chatham laboratory, for the statistical analysis of the data.

## REFERENCES

1. Albritton, E. C. Standard values in nutrition and metabolism. W. B. Saunders Co., Philadelphia, Penna. 1954.
2. Brodelli, A. P., P. E. Stricker, and H. C. Phillips. Extent and cost of spraying and dusting on farms, 1952. U.S. Dept. Agr., Agr. Res. Service Sta. Bull. 156, 1955.
3. Chamberlin, T. R. Further tests of insecticides to control meadow spittlebugs in alfalfa. J. Econ. Entomol. 43:888-891. 1950.
4. Chamberlin, T. R., and J. T. Medler. Tests of insecticides against the meadow spittlebug on seed alfalfa. J. Econ. Entomol. 42:653-656. 1949.
5. Gyrisco, G. C., and D. S. Marshall. The control of insects of alfalfa and red clover in New York. J. Econ. Entomol. 43:438-443. 1950.
6. Kerr, T. W., Jr., and I. H. Stuckey. Insects attacking red clover in Rhode Island and their control. J. Econ. Entomol. 49:371-375. 1956.
7. Marshall, D. S., and G. C. Gyrisco. Control of the meadow spittlebug on forage crops. J. Econ. Entomol. 44:289-293. 1951.
8. Medler, J. T. Control of common alfalfa insects in Wisconsin. J. Econ. Entomol. 48:718-723. 1955.
9. Ontario Department of Agriculture. Agricultural statistics for Ontario, 1953. Statistics and Publications Branch, Toronto. 1954.
10. Wilson, M. C. Organic insecticides to control alfalfa insects. J. Econ. Entomol. 42:496-498. 1949.

# THE USE OF POLLEN INSERTS FOR TREE FRUIT POLLINATION<sup>1</sup>

G. F. TOWNSEND,<sup>2</sup> R. T. RIDDELL<sup>3</sup> AND M. V. SMITH<sup>4</sup>

*Ontario Agricultural College, Guelph, Ontario*

[Received for publication April 30, 1957]

## ABSTRACT

Tests conducted in the Niagara and Georgian Bay fruit-growing areas for the past 3 years have indicated that pollen distributed from inserts placed at the entrances of honeybee colonies is effective as a means of cross-pollinating tree fruits. This technique is particularly useful in seasons when honeybee foraging activities are limited by unfavourable weather, or in plantings where a sufficient number of pollinator varieties for natural cross-pollination have not been provided.

## INTRODUCTION

The opportunity for cross-pollination of tree fruits is often seriously jeopardized by the lack of suitable pollinator varieties. Several investigators have attempted to circumvent this shortcoming by setting out for orchard pollination honeybee colonies which have been fitted with a special pollen-dispensing device. Foraging bees leaving the hive were forced to walk through a tray containing viable pollen from a compatible variety, in the hope that they would carry enough of this pollen to the blossoms to bring about cross-pollination.

A type of pollen dispenser, or insert, as it is currently called, was devised and tested by Burrell and King (2). Their results were inconclusive. Overley and O'Neill (9) carried out tests with pollen inserts on apples, but were unable to demonstrate their effectiveness. Kremer (7) and Antles (1) each described and tested inserts which they hoped would prove satisfactory for pollen distribution by honeybees. However, with conclusive evidence as to the effectiveness of inserts still lacking, Griggs, Vansell and Iwakiri (4) undertook a study in California, using almond and sweet cherry trees, which they covered with tents of cheesecloth netting to exclude outside pollinators. In each tent they placed a hive of bees, fitted with an insert. They obtained such poor fruit sets that they concluded this means of effecting cross-pollination was not satisfactory.

Then Karmo and Vickery (6), after several seasons of testing pollen inserts on apples in the Annapolis Valley of Nova Scotia, reported very satisfactory results. This led the authors to investigate inserts as a means of cross-pollination of tree fruits in Ontario.

## PROCEDURE AND METHODS

### *Preliminary Tests*

First the Kremer and Harwood-Antles inserts were tested; each of these had obvious disadvantages. The Kremer insert was very wasteful of pollen, and outgoing bees soon fanned the pollen back into the hive, where it was lost. The rather narrow Harwood-Antles insert, which covered only part of the hive entrance, permitted too many bees to leave the hive

<sup>1</sup>This work was carried out to aid in honeybee pollination studies as part of the program of the Tree Fruit Research Committee in Ontario.

<sup>2</sup>Professor and Head of Department of Apiculture.

<sup>3</sup>Assistant Professor, Department of Botany.

<sup>4</sup>Assistant Professor, Department of Apiculture.

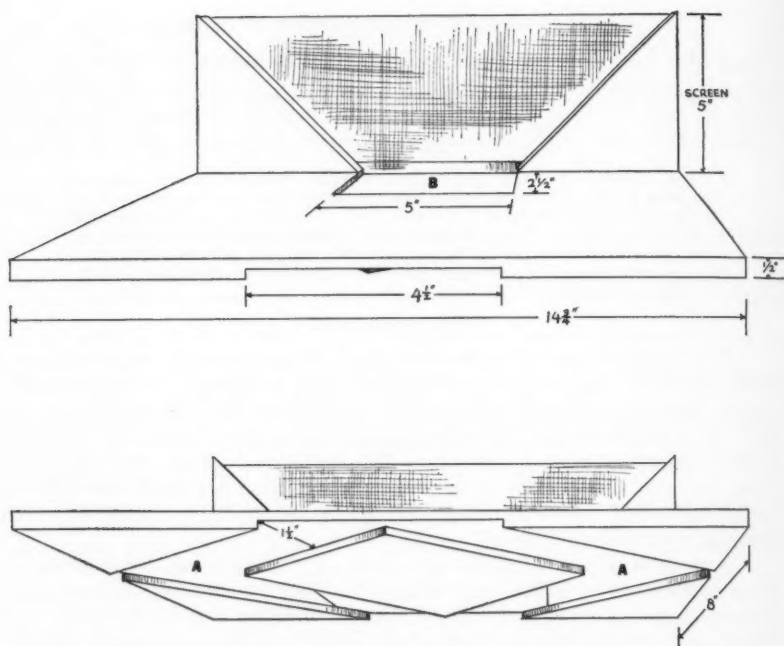


FIGURE 1. Modified Nova Scotia Agricultural College pollen insert. *Upper*—top view from rear, showing the pollen tray (B). *Lower*—bottom view from rear, showing modified passage-ways for returning honeybees (A).

without walking through the pollen tray. Next, the N.S.A.C. (Nova Scotia Agricultural College) insert, developed by Karmo, was tried. This appeared to be more satisfactory in most respects, but the entrance for the incoming bees, which was at one side of the insert, often resulted in a congestion of returning foragers. To overcome this difficulty, the N.S.A.C. insert was modified to provide a centre entrance for returning bees. This insert, shown in Figure 1, is the one which has proved most satisfactory and upon which the authors' observations were based.

An attempt was made in the spring of 1953 to test the efficiency of pollen inserts under controlled conditions, similar to the method used by Griggs *et al.* (4). A young sweet cherry tree (*Heidelfingen* × *Victor*) was enclosed in a tent before the buds had opened. When the tree was in bloom a colony of bees fitted with a pollen insert was placed under the tent. The pollen tray was replenished at frequent intervals for a 2-day period with hand-collected Black Tartarian pollen diluted with an equal part of lycopodium spores.

Bees were observed to work on the blossoms. However, before the test was completed the temperature rose above 80° F. and it became so hot inside the tent that the blossoms turned brown and withered. The observed fruit set of 2.5 per cent was believed to be largely due to excessive temperature.



The following season, a different approach was used in order to determine whether the mechanics of pollen distribution from inserts was as sound in practice as in theory. A mixture consisting of three parts of pollen with one part of a fluorescent powder\* was placed in the insert trays. This powder, which fluoresced a bright green in the solid state, served as a tracer to identify blossoms which had received pollen carried from the inserts by the bees. By examining the orchard after dark with a portable ultra-violet light it was possible to determine the effectiveness of the insert-fitted colonies as pollen distributors.

Tests carried out in sweet cherry, pear and apple orchards showed beyond a doubt that the bees were carrying the pollen to the trees and depositing it on the blossoms. Under favourable flight conditions, two colonies per acre, with inserts maintained in position for a short a period as 2 hours, resulted in 75 to 100 per cent of the trees in the orchard receiving the marked pollen.

Wild bees, collected on several occasions from blossoms in orchards where fluorescent pollen was being distributed, were found to have traces of the marker on their legs and bodies. Particles of the fluorescent powder were also found at the entrances of nearby honeybee colonies which had not been used as pollen distributors. This indicates that the pollen carried from the insert trays to the blossoms may be further distributed by subsequent visitations of other insects, thereby increasing the efficiency of this method of cross-pollination.

Having thus demonstrated the soundness of the principle of using pollen inserts for the distribution of pollen, it remained to carry out a field test of this means of cross-pollination.

#### *Practical Tests*

A 16-acre commercial orchard located in the Georgian Bay area was used for field testing the efficiency of pollen inserts. This 18-year-old orchard was planted to Bartlett pears with Kieffer trees as pollinators. A few scattered replants of Flemish Beauty, Anjou and Bosc varieties were added, but the orchard had not produced a commercial crop of fruit.

Several years earlier, part of the experimental orchard had been divided into plots for replicated experiments in fertility and orchard management. Thus there were trees which were in a high state of vigour, as well as trees with low and intermediate degrees of vigour. A total of 374 trees in this orchard were under close observation with respect to blossom production, fruit set and fruit yield, size and quality. However, the low fruit set and the lack of uniformity of fruit set throughout the orchard had introduced so much variability into the results that it was imperative to find some means of overcoming this factor.

In the spring of 1955, and again in 1956, 32 standard 2-storey colonies of average strength were moved into the orchard. The bees were moved after the trees were in bloom in order to obtain maximum foraging populations in the orchard for which they were intended. Groups of 4 or 5 colonies were placed throughout all parts of the orchard in order to give as uniform coverage as possible. As soon as the bees began to fly actively,

\*Fluorescent No. 2266, New Jersey Zinc Co., New York, N.Y.

inserts were placed in position at the colony entrances. It should be noted that the bees from particularly strong colonies may cluster at the front of the hive when their entrance is restricted by the addition of an insert. This can be prevented by raising the cover and screening the top of the colony to provide additional ventilation.

In the 1955 experiments, bees placed in the orchard at 11.00 a.m. were flying strongly by 1.30 p.m., when the inserts were placed in position. Every 20 to 30 minutes one teaspoon of fresh pollen was placed in the insert tray of each colony until flight activity began to drop off at 4.00 p.m. During the flight period an average of 12 bees per tree was recorded. One-half ounce of pollen per acre, diluted three times with lycopodium, was dispensed during this time. It then turned cold and wet, and the bees were unable to fly again for the remainder of the blooming period. Thus the only pollination this orchard received was accomplished during the one 2½-hour interval when the inserts were in place.

The following year, the bees were moved on a cool wet day and the weather remained this way for the following 4 days. After their long confinement the bees took full advantage of intermittent sunny periods on the fifth day, and, although the temperature only rose to 55° F., 15 bees per tree were counted. Pollen inserts were in use from 1.30 to 4.00 p.m., and for an additional 2 hours the following afternoon. One ounce of pollen per acre, diluted five times with lycopodium powder, was dispensed during these two periods.

Hand-collected standard Winter Nelis pollen, imported from the United States, was used in these tests. The pollen was stored in a deep freeze at 0° F., and was transported to and kept in the orchard in sealed jars placed in large thermos jugs of ice. Pollen viability, checked both before and after the tests, ranged between 85 and 92 per cent.

Pollen pellets collected by honeybees can be trapped quite readily at the hive entrance, and it has been suggested that this might be a more

TABLE 1.—BARTLETT PEAR FRUIT SET IN RELATION TO POLLINATOR PROXIMITY

Pollinator	Per Cent of Blossom Clusters Developing Fruit					
	Next to pollinator		One tree removed		Two trees removed	
	Without inserts	With inserts	Without inserts	With inserts	Without inserts	With inserts
Kieffer I	22	54	17	47	8	51
Kieffer II	25	51	10	53	7	53
Kieffer III	25	49	14	53	2	57
Anjou I	19	38	10	33	12	40
Anjou II	27	61	6	74	3	52
Anjou III	22	53	18	52	5	53
Flemish Beauty I	21	47	21	70	5	63
Flemish Beauty II	24	66	20	73	5	78
Average	23	52	15	57	6	56

NOTE: *Without inserts*—Average fruit set data for 1952, 1953 and 1954 seasons  
*With inserts* —Average fruit set data for 1955 and 1956 seasons

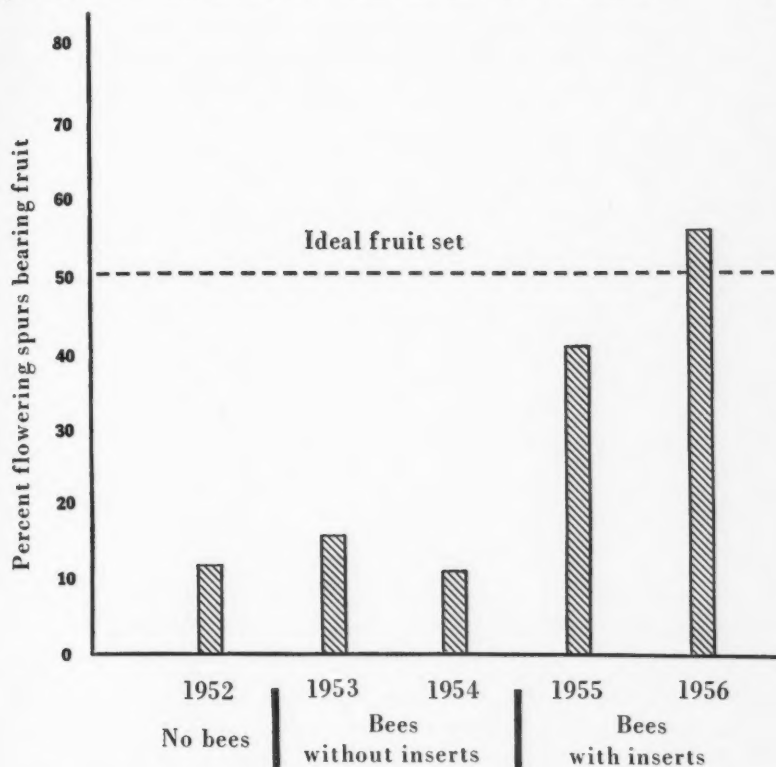


FIGURE 2. Five-year comparison of fruit set in Bartlett pear test orchard.

economical source of insert pollen. Singh and Boynton (10), Kremer (8) and Griggs *et al.* (3) (5) have all carried out viability tests on bee-collected fruit pollens. On the whole, they found that pellet pollen loses its viability more rapidly and produces a lower fruit set than does hand-collected pollen. In addition, the purity to species of bee-collected pollen is often quite variable. For these reasons, hand-collected pollen is to be preferred for most efficient insert use.

#### RESULTS

Pronounced increases in fruit set and in uniformity of set throughout the orchard were obtained with the aid of insert-equipped honeybee colonies. This practically eliminated one of the major sources of variation in the fertility and orchard management experiments.

The data in Table I were selected to illustrate the fruit set on trees from different plots when bees alone were used and when pollen inserts were added. The figures in this table are percentages derived from at least 1800 counts of blossom clusters on selected branches on 4 trees. The figures in the first, third and fifth columns represent the effect of natural pollen distribution from the pollinator varieties on fruit set on the surround-

ing trees during the 1953 and 1954 seasons. These percentages remained quite stable, regardless of whether the tree was in the heavy bearing year or in the alternate year of low blossom production. The rapid decline in fruit set percentage, particularly on trees two rows distant from the pollen source, indicates that cross-pollination was being imperfectly carried out. After the pollen inserts were used there was a marked increase in fruit set per tree, and the resulting fruit set from all trees was quite uniform, regardless of the distance from a pollinator variety.

A similar orchard owned by the same grower and situated a mile away did not show any increase in fruit set or in uniformity of set during 1955 or 1956. The average fruit set in this orchard ranged from 8 to 14 per cent over the 5-year period. Thus weather conditions did not contribute significantly to the increased fruit set.

Vigorous high-yielding pear trees require 50 per cent of the blossom clusters to bear fruit if the maximum marketable crop is to be produced. In Figure 2, the fruit set data for the past 5 years are compared to this hypothetical ideal yield. Note that no increase in fruit set was brought about, even when colonies were moved into the orchard, until pollen inserts were used. The highest yielding plots in this orchard, which produced an average of 4.25 tons of fruit per acre in 1954, showed an increase to 9.5 tons in 1956.

#### CONCLUSIONS

The efficient use of pollen inserts on honeybee colonies set out for orchard pollination requires attention to a number of details. Careful consideration must be given to timing of the application, uniform distribution of colonies, number of colonies, selection of a suitable pollen, care of the pollen, frequent filling of the insert trays, and to the foraging activities of the bees themselves. In addition, only healthy well-fed trees can be expected to respond to this treatment.

In the Bartlett pear orchard under study, where all these criteria were met, pollen inserts were responsible for pronounced increases in fruit set.

#### REFERENCES

1. Antles, L. C. New methods in orchard pollination. *Amer. Bee J.* 93(3):102-103. 1953.
2. Burrell, A. B., and G. E. King. A device to facilitate pollen distribution by bees. *Proc. Amer. Soc. Hort. Sci.* 28:85-86. 1932.
3. Griggs, W. H., and G. H. Vansell. The use of bee-collected pollen in artificial pollination of deciduous fruits. *Proc. Amer. Soc. Hort. Sci.* 54:118-124. 1949.
4. Griggs, W. H., G. H. Vansell, and B. T. Iwakiri. The use of beehive pollen dispensers in the pollination of almonds and sweet cherries. *Proc. Amer. Soc. Hort. Sci.* 60:146-150. 1952.
5. Griggs, W. H., G. H. Vansell, and J. F. Reinhardt. The germinating ability of quick frozen, bee-collected apple pollen stored in a dry-ice container. *J. Econ. Entomol.* 43:549. 1950.
6. Karmo, E. A., and V. R. Vickery. The place of honeybees in orchard pollination. N.S. Dept. Agriculture and Marketing, Mimeo. Circular Service 67. 1954.
7. Kremer, J. C. Traps for the collection and distribution of pollen in orchards. *Mich. Agr. Expt. Sta. Quart. Bull.* 31 1; pp. 12-21. 1948.
8. Kremer, J. C. Germination tests of the viability of apple pollen gathered in pellets. *Proc. Amer. Soc. Hort. Sci.* 53:153-157. 1949.
9. Overley, F. L., and W. J. O'Neill. Experiments with the use of bees for pollination of fruit trees. *Proc. Washington State Hort. Assoc.* 203-214. 1946.
10. Singh, S., and D. Boynton. Viability of apple pollen in pellets of honeybees. *Proc. Amer. Soc. Hort. Sci.* 53:148-152. 1949.

# INHERITANCE OF REACTION TO *USTILAGO HORDEI* (PERS.) LAGERH. IN CULTIVATED BARLEY<sup>1</sup>

S. A. WELLS

Canada Department of Agriculture, Lethbridge, Alberta

[Received for publication April 8, 1957]

## ABSTRACT

Results from inoculated  $F_1$ ,  $F_2$ , and  $F_3$  generations of diallel crosses between five resistant varieties and one susceptible variety of barley (Junior) indicated that four different genes for resistance to *Ustilago hordei* were involved. A dominant gene for resistance appears to be common to Titan, O.A.C. 21, Ogalitsu, and Anoidium. Anoidium carries a second dominant gene for resistance (designated *Uh2*), Ogalitsu an additional recessive (designated *uh3*), and Jet, a single recessive (designated *uh4*). Interpretation of the results was complicated by skewed  $F_2$  and  $F_3$  distributions resulting from seedling mortality of severely infected plants. Chi-square tests for independence of morphological characters indicated that a gene for awn barbing is located in linkage group I. No association was found between inheritance of covered smut reaction and inheritance of number of kernel rows, lemma teeth, lemma and pericarp colour, hull adherence, aleurone colour, awn barbing, length of rachilla hairs, or rachis pubescence.

## INTRODUCTION

Information on the inheritance of reaction to *Ustilago hordei* (Pers.) Lagerh., incitant of covered smut of barley, is rather limited. Several workers have studied the problem, but most have been unable to obtain sufficiently high infection of susceptible material to classify their results in a satisfactory manner. Johnston (5) studied the reaction of  $F_3$  lines from a cross between Glabron (resistant) and Trebi (moderately susceptible) to the collection of *U. hordei* that he used. Segregation was not sufficiently clear to establish the number of genes involved. Woodward and Tingey (14) inoculated two  $F_2$  hybrid families from crosses between the susceptible variety Winter Club and the resistant varieties Union Beardless and Colorado 3063. The amount of infection obtained was too low to permit a genetic interpretation of the results. Pugsley and Vines (6) studied the reaction of  $F_3$  progenies from the cross between Cape (susceptible) and Kwan (resistant) to a collection similar to Tapke's race 5 (12). Their results could not be interpreted on a genetic basis. Recently, however, Shands (10) found that resistance to race 6 of *U. hordei* was conditioned by one major factor pair in the crosses Chevron  $\times$  Brachytic and Colsess  $\times$  Brachytic.

Several workers have reported reduced stands from dehulled barley seeds inoculated with *U. hordei*. Faris (2) obtained higher infection percentages under field conditions from barley that was hand-dehulled before inoculation with dry spores, than from hulled seed. However, there was considerable seedling distortion and poor emergence from hand-dehulled seed. Briggs (1), and Woodward and Tingey (14), also encountered reduced stands from inoculated, hand-dehulled seed. Johnston (5) demonstrated that reduced stands from dehulled seeds were the result of increased severity of infection by the pathogen. He obtained poorer

<sup>1</sup> Contribution No. 216 from the Cereal Crops Division, Experimental Farms Service. Based on a thesis submitted to the School of Graduate Studies, University of Alberta, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

TABLE 1.—CONTRASTING CHARACTERS OF THE VARIETY JUNIOR AND OTHER PARENTAL VARIETIES  
(Where no information is given, the variety concerned does not differ from Junior for the character listed)

Characters	Junior	O.A.C. 21	Titan	Ogalitsu	Anodium	Jet
Row number	Six-rowed	—	—	—	—	Two-rowed
Lemma	Toothed	—	Untoothed	—	Untoothed	—
Lemma and pericarp	White	—	—	—	—	Black
Hull adherence	Hulless	Hulled	Hulled	Hulled	Hulled	—
Aleurone	White	Blue	—	—	Blue	—
Awns	Rough	—	Smooth	Semi-smooth	Smooth	—
Rachilla hairs	Long	Short	—	Heterogeneous	Short	Short
Rachis edges	Pubescent	—	Non-pubescent	—	—	—
Covered smut (race 6)	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant

stands from inoculated than from uninoculated hand-dehulled seed. This was confirmed in experiments by the author.<sup>1</sup> Furthermore, stands from hand-dehulled seeds were lower than stands from hulled seeds, probably as a result of mechanical injury during the dehulling process. Shands (10) found a direct relationship between smut reaction and stand. Averages of classifiable plants for Chevron  $\times$  Brachytic  $F_3$  progenies within resistant, segregating, and susceptible smut classes were 78.0, 72.4, and 62.4 per cent, respectively.  $F_3$  progenies of Colless  $\times$  Brachytic acted similarly.

Information on the inheritance of resistance to covered smut, and on linkage relationships with morphological characters, would be useful to the barley breeder. The present study was undertaken in an attempt to discover what genes for resistance are present in certain varieties; to find out whether or not a number of such genes can be combined in a single variety; and to investigate linkage relationships between resistance and morphological characters.

#### MATERIALS AND METHODS

Tapke (12) reported that race 6 of *U. hordei* is usually predominant where varieties of the Manchuria-Oderbrucker barley type prevail. Most Canadian barleys are of this type. For this reason, and because race 6 gave clear-cut distinctions between resistance and susceptibility among the proposed parent varieties, it was used in this study. Tapke's differential hosts were used to check the behaviour of race 6 under Lethbridge conditions. One test was sown in the field in 1953 in two replicates with 100 seeds in each plot. A second test was sown in the greenhouse in the spring of 1954 along with crosses that involved Junior, the susceptible parent. Ten replicates were sown with 30 seeds in each plot. In both tests hulled varieties were hand-dehulled before inoculation with dry spores.

The parents of the hybrid populations studied were O.A.C. 21 (C.A.N.\* 1086), Titan (C.A.N. 1164), Ogalitsu (C.I.\*\*7152), Anodium (C.I. 7269),

<sup>1</sup>Unpublished.

\* C.A.N. Canadian Accession Number.

\*\* C.I. Cereal Investigations, U.S. Dept. Agr. Accession Number.



Jet (C.I. 967) and Junior (C.A.N. 766). Differences in morphological characters between the covered smut resistant varieties and Junior, the susceptible variety, are shown in Table 1.

Diallel crosses were made between the six parents, resulting in 15 crosses. Sufficient crossed seed from each population to produce an adequate  $F_1$  was sown without inoculation and the remainder saved for smut reaction. Similarly, part of the seed from each  $F_1$  plant was sown without inoculation and the remainder saved for smut reaction. The resulting  $F_2$  plants were harvested and threshed individually to provide seed for the  $F_3$  generation. Thus, the  $F_3$  of each population consisted of descendants of the same families that were used in the  $F_2$ . All hybrid material was threshed by hand.

In crosses involving Junior,  $F_2$  plants were classified for those morphological characters by which the parents differed. Their identity was retained through the  $F_3$  generation and classifications were checked by examining the  $F_3$  progenies. This procedure was especially valuable in classifying for aleurone colour. The classification of  $F_2$  plants was not reliable for this character, and  $F_2$  results were not used unless corroborated by the  $F_3$ .

The seed was dehulled by peeling the lemma back from the germ end of the seed in order to expose the embryo. Thirty seeds of each lot to be inoculated were dehulled in this manner and placed in a small coin envelope. A 1:1 mixture of chlamydospores and French chalk was introduced into each envelope and the whole shaken vigorously to effect complete coverage of all seeds. The 30 seeds in each envelope were sown in a single row.

The  $F_1$ ,  $F_2$ , and  $F_3$  generations of each cross were grown under comparable conditions, together with the appropriate parents as checks. The crosses involving Junior were sown to a depth of 3 inches in ground beds in the greenhouse, in rows 30 inches long and 3 inches apart. The  $F_1$  populations,  $F_3$  lines, and parents were sown in individual rows, while the  $F_2$  of each cross occupied several rows. The resistant parent involved in each cross occurred in every fifteenth row of that cross and Junior in every thirtieth row throughout all crosses. Soil temperatures were maintained at 60°F. at night. Daytime temperatures were higher but were kept below 80°F. Little variation in temperature was observed between beds.

Crosses between resistant varieties were grown under irrigation in the field in rows 8 feet long and 1 foot apart. Parental checks were sown about every twentieth row. As a check on the degree of infection occurring in susceptible material, Junior was sown every hundredth row. Seeding was delayed until June 25, when soil temperatures ranged between 57°F. and 67°F. A few days of cool weather followed. The minimum temperature recorded was 51°F. in the early morning of June 29, with a maximum of 55°F. that day. Soil temperatures in the field were probably too low for maximum infection during the germination period.

After heading, the plants in all rows were pulled and counted. The total number of plants and the number infected were recorded for each row. The percentage of infected plants was calculated to the nearest whole number.

## EXPERIMENTAL RESULTS

*Reaction of Differential Hosts*

The results obtained from inoculations of eight differential hosts with the race of *U. hordei* used in this study are presented in Table 2. The high proportions of infected plants in susceptible varieties indicate that conditions in the greenhouse were favourable for maximum expression of the disease. The reactions of the differential hosts to this race were similar to those obtained to race 6 by Tapke (12).

*Inheritance of Reaction to Covered Smut in Crosses with Junior*

The reactions of parents,  $F_1$ , and  $F_2$  generations of crosses involving Junior are shown in Table 3. The distributions of parental rows,  $F_2$  rows, and  $F_3$  progenies by 5 per cent infection class intervals are shown in Table 4.

TABLE 2.—PERCENTAGE OF INFECTED PLANTS IN BARLEY DIFFERENTIAL HOSTS INOCULATED WITH *U. hordei* RACE 6

C.I. No.	Variety	Field, 1953	Greenhouse, 1954
1248	Excelsior	0.0	0.0
1312	Himalaya	0.0	0.0
531	Hannuchen	44.0	94.8
923	Lion	2.6	47.4
595	Nepal	0.0	0.0
934	Odessa	51.9	99.1
1330	Pannier	0.0	0.0
936	Trebi	16.3	90.0

TABLE 3.—PERCENTAGE OF INFECTED PLANTS OF PARENTS,  $F_1$ , AND  $F_2$  OF CROSSES INOCULATED WITH RACE 6 OF *U. hordei* AND GROWN IN THE GREENHOUSE

Material	Total plants	No. Healthy	No. Infected	% Infected
Junior × O.A.C. 21 $F_1$	18	18	0	0.0
Junior × O.A.C. 21 $F_2$	349	312	37	10.6
Junior	149	33	116	77.8
O.A.C. 21	369	366	3	0.8
Junior × Titan $F_1$	23	23	0	0.0
Junior × Titan $F_2$	290	241	49	16.9
Junior	172	53	119	69.2
Titan	387	387	0	0.0
Junior × Ogalitsu $F_1$	22	21	1	4.5
Junior × Ogalitsu $F_2$	395	379	16	4.0
Junior	154	38	116	75.3
Ogalitsu	333	333	0	0.0
Junior × Anoidium $F_1$	15	15	0	0.0
Junior × Anoidium $F_2$	337	322	15	4.4
Junior	188	51	137	72.9
Anoidium	360	359	1	0.3
Junior × Jet $F_1$	13	5	8	61.5
Junior × Jet $F_2$	249	158	91	36.5
Junior	173	33	140	80.9
Jet	258	256	2	0.8

TABLE 4.—DISTRIBUTION OF PARENTAL ROWS, F<sub>2</sub> ROWS, AND F<sub>3</sub> PROGENIES, IN 5 PER CENT INFECTION CLASS INTERVALS, OF CROSSES INOCULATED WITH RACE 6 OF *U. hordei* AND GROWN IN THE GREENHOUSE

Material	0	1 -5	6 -10	11 -15	16 -20	21 -25	26 -30	31 -35	36 -40	41 -45	46 -50	51 -55	56 -60	61 -65	66 -70	71 -75	76 -80	81 -85	86 -90	91 -95	96 -100	Total rows
Junior X O.A.C. 21 F <sub>2</sub>	2	4	4	1	2	1	0	0	1	7	5	2	0	1	6	3	2	3	1	0	1	15
Junior X O.A.C. 21 F <sub>2</sub>	46	16	19	16	19	3	7	5	1	7	1	0	0	0	1	0	2	3	0	1	1	163
Junior O.A.C. 21	13	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	13
Junior X Titan F <sub>2</sub>	1	2	1	2	2	3	1	2	5	—	7	3	6	2	6	4	3	2	1	2	0	14
Junior X Titan F <sub>2</sub>	60	7	19	24	17	10	5	3	1	0	1	0	1	0	1	3	2	2	1	1	0	186
Junior Titan	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15
Junior X Ogallitsu F <sub>2</sub>	7	6	4	1	7	4	3	6	4	7	1	5	1	1	0	0	0	1	0	—	—	18
Junior X Ogallitsu F <sub>3</sub>	83	17	20	30	7	—	—	—	—	—	—	—	—	0	2	2	1	2	0	0	1	190
Junior Ogallitsu	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16
Junior X Anoidium F <sub>2</sub>	3	8	2	0	1	3	7	1	1	4	2	3	1	—	—	—	—	—	—	—	—	14
Junior X Anoidium F <sub>2</sub>	82	17	23	14	9	—	—	—	—	—	0	0	1	1	1	0	0	2	0	1	—	167
Junior Anoidium	13	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14
Junior X Jet F <sub>2</sub>	—	—	—	—	1	3	2	3	1	2	0	1	3	5	0	1	2	3	0	0	1	16
Junior X Jet F <sub>2</sub>	35	15	21	13	14	9	7	8	7	4	5	2	2	—	—	3	2	1	3	1	—	154
Junior Jet	13	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	12
Junior (all crosses)	—	—	—	—	—	—	—	—	1	1	2	0	3	1	6	8	7	8	4	3	4	48

As in previous investigations (5, 10), there was evidence in this study that infection by *U. hordei* was responsible for some seedling mortality. The average stand of the rows of each of the resistant parents was higher than that of comparable rows of Junior. The average stand of the check rows of all resistant parents was 73 per cent, while that of the check rows of the susceptible parent, Junior, was 58 per cent. This occurred in spite of the fact that the seed of four of the resistant parents had been hand-dehulled, a procedure that usually results in some mechanical injury, and therefore reduced stands. The seed used for the check rows of Junior was threshed by hand to avoid mechanical injury, and required no dehulling. This evidence suggests that stands of Junior were materially reduced as a result of infection by the pathogen.

If seedling mortality of Junior was caused by smut infection,\* the susceptible  $F_3$  lines should have been affected. In that event, there should have been an inverse relationship between stand and infection in the  $F_3$ . Accordingly, percentage stand was calculated for each  $F_3$  line. To eliminate zero values, 0.1 was added to each infection percentage. Percentage values for stand and infection were converted to  $\sin^2\theta$ , and a correlation coefficient calculated for each cross. The following results were obtained:

Cross	r.
Junior $\times$ O.A.C. 21	-0.186*
Junior $\times$ Titan	-0.258**
Junior $\times$ Ogalitsu	-0.095
Junior $\times$ Anoidium	-0.256**
Junior $\times$ Jet	-0.265**

\*  $P < 0.05$

\*\*  $P < 0.01$

It is apparent that some susceptible plants failed to emerge. The negative association between stand and infection in the cross Junior  $\times$  Ogalitsu, although not statistically significant, indicates that there was probably some loss of susceptible seedlings. Since seedling mortality might have disturbed the ratios, its probable effect was considered in the genetic interpretations of the results.

Interpretations were based largely on  $F_3$  results. In chi-square tests for goodness of fit of observed to expected  $F_2$  results, the expected ratios were corrected for escapes on the basis of the reaction of the susceptible parent, Junior. For example, the observed  $F_2$  ratio of the cross Junior  $\times$  Titan was 241 healthy to 49 infected plants. Assuming a single dominant gene for resistance, the theoretical ratio would be 217.5 resistant to 72.5 susceptible plants. However, only 69.2 per cent of the plants of Junior were infected. Therefore, only about that percentage of the  $F_2$  plants of susceptible genotype would be expected to be infected. When 30.8 per cent of the susceptible class is transferred to the resistant class, the expected ratio becomes 239.8 healthy to 50.2 infected plants. This ratio is very similar to the actual ratio.

In interpreting the  $F_3$  distributions of all crosses shown in Table 4 except Junior  $\times$  Ogalitsu, lines free from smut were classed as resistant, conforming with the reactions of the resistant parents. Although a few of the resistant lines might be expected to contain some smut, the number

of such lines would be small, and in most crosses would be compensated for by a similar number of segregating lines that might be included in the resistant category.

#### *Junior* × *O.A.C. 21*

The resistant reaction of the  $F_1$  of this cross and the low proportion of infected plants in the  $F_2$  (Table 3) indicate that the resistance of *O.A.C. 21* is dominant. The distribution of the  $F_3$  progeny rows (Table 4) suggests a single dominant gene for resistance.

In the  $F_3$ , progeny rows containing from 1 per cent to 25 per cent infected plants were classed as segregating, and those containing more than 25 per cent as susceptible. Since all but one of the  $F_2$  rows contained less than 25 per cent infected plants, this was chosen as the point which would separate segregating from susceptible lines with a minimum of misclassification. Furthermore, this point coincides with a low point in the distribution of  $F_3$  lines resulting from breaks in the distributions of four of the five individual  $F_3$  families. Forty-six  $F_3$  lines were classified as homozygous resistant, 73 as segregating, and 44 as susceptible. This ratio fits the expected 1:2:1 ratio (chi-square = 1.82 and  $P = 0.40$ ).

The  $F_2$  ratio of 312 healthy to 37 infected plants did not fit the hypothesis, even when the expected ratio was corrected for escapes on the basis of the reaction of *Junior*. Mortality of infected seedlings would be expected to reduce the proportion of smutted plants in the  $F_2$ , and probably accounts for the lack of agreement between observed and expected ratios.

#### *Junior* × *Titan*

The  $F_1$  of this cross was resistant (Table 3), indicating that the resistance of *Titan* is dominant. The percentage of infected plants in the  $F_2$  approached the expected where a single dominant gene governs resistance. The distributions of the  $F_2$  rows and  $F_3$  lines shown in Table 4 support this assumption.

$F_3$  lines were classified as resistant, segregating, or susceptible. Lines containing from 1 per cent to 35 per cent infected plants were classed as segregating, corresponding to the distribution of the segregating  $F_2$  rows. Lines containing more than 35 per cent infected plants were classed as susceptible. In this manner 60 lines were classified as resistant, 85 as segregating, and 41 as susceptible. This ratio fits the expected 1:2:1 ratio (chi-square = 5.26 and  $P = 0.10$ ).

The expected  $F_2$  ratio, corrected for escapes, was 239.8 healthy to 50.2 infected plants. This is close to the actual ratio (chi-square = 0.035 and  $P = 0.90$ ).

#### *Junior* × *Ogalitsu*

The infected plant in the  $F_1$  row of the cross *Junior* × *Ogalitsu* (Table 3) probably came from a self-fertilized floret of *Junior*. The low percentage of infected plants in the  $F_2$  (Table 3), as well as the distribution of the  $F_2$  rows and  $F_3$  progenies (Table 4), indicate more than one factor for resistance. It was assumed that two genes, one dominant and the other recessive, governed the inheritance of smut reaction.

On this basis, the following proportions of progenies are expected in the  $F_3$ :

Frequency	Class
7	homozygous resistant
4	segregating 13 resistant to 3 susceptible
2	segregating 3 resistant to 1 susceptible
2	segregating 1 resistant to 3 susceptible
1	homozygous susceptible

In classifying the  $F_3$  progenies no attempt was made to separate resistant lines, lines segregating 13:3, or those segregating 3:1, since these classes could be expected to overlap. Similarly, lines segregating 1:3 were not separated from susceptible lines, again because of overlapping. Lines containing up to 25 per cent infected plants were placed in the first category and those containing more than 25 per cent infected plants in the second. The point chosen is a low point in the distribution of the  $F_3$  lines near the theoretical point of separation. Lines segregating 1:3 should be distributed at a lower range than rows of Junior, since they would be less susceptible. Furthermore, seedling mortality of susceptible plants would tend to move the distribution of such lines toward the less susceptible classes. There were 161 lines in the first category and 29 in the second. The test for goodness of fit of these results to the expected 13:3 ratio indicates close agreement ( $\chi^2 = 1.51$  and  $P = 0.20$ ).

The  $F_2$  ratio of 379 healthy to 16 infected plants did not fit the expected 13:3 ratio, even when the latter was corrected for escapes on the basis of the reaction of Junior. This is not surprising since seven of the 18  $F_2$  rows were free from smut. Seedling mortality could account for the small number of infected plants.

#### *Junior* $\times$ *Anoidium*

The reactions of the  $F_1$  and  $F_2$  of this cross (Table 3) indicate that resistance is dominant. The distribution of the  $F_3$  progeny rows (Table 4) indicates that more than one gene is involved. It was assumed that *Anoidium* carries two dominant genes for resistance. Thus, the following proportions of  $F_3$  progenies would be expected:

Frequency	Class
7	homozygous resistant
4	segregating 15 resistant to 1 susceptible
4	segregating 3 resistant to 1 susceptible
1	homozygous susceptible

The  $F_3$  progeny rows were classified as resistant, segregating, or susceptible. No attempt was made to separate the two groups of segregating lines, since considerable overlapping would be expected. Lines containing from 1 per cent to 30 per cent infected plants were classed as segregating, and lines with more than 30 per cent diseased plants were classed as susceptible. The separation between segregating and susceptible lines was based on the fact that there was a definite break in the distributions of two of the three individual  $F_3$  families at the 30 per cent point, which is near the theoretical separation point. Classified in this manner the observed ratio was 82 resistant to 73 segregating to 12 susceptible lines. This agrees with the theoretical 7:8:1 ratio ( $\chi^2 = 2.65$  and  $P = 0.30$ ).



The  $F_2$  data from this cross support the hypothesis that resistance was governed by two dominant genes. The observed ratio was 322 healthy to 15 infected plants. The expected ratio, corrected for escapes on the basis of the reaction of Junior, was 321.6:15.4 (chi-square = 0.01 and  $P = 0.90$ ).

#### *Junior × Jet*

The distribution of  $F_3$  progenies (Table 4) suggests that a single dominant gene governed resistance in this cross. However, the  $F_1$  was susceptible, and infection in the  $F_2$  too high to be accounted for on the basis of a dominant gene.

In classifying the  $F_3$  lines, those free from smut were considered resistant, and those containing smut as segregating or susceptible. No separation was made between segregating and susceptible lines. Thus, 35 lines were classed as resistant and 119 as segregating or susceptible. This ratio fits the theoretical 1:3 ratio (chi-square = 0.43 and  $P = 0.50$ ).

It is probable that only one gene for resistance was involved in this cross. High infection in the  $F_1$  and  $F_2$  indicates that the gene is not dominant. It is possible that Jet carries a single recessive gene for resistance, and that the distribution of  $F_3$  lines was skewed toward the resistant classes by seedling mortality of severely infected plants. The highly significant negative correlation between stand and infection ( $r = -0.265$ ) supports this hypothesis. Susceptible plants that escaped infection would tend also to skew the distribution toward the resistant classes.

The  $F_2$  data did not fit a single gene ratio, even when corrected for escapes on the basis of the reaction of Junior.

#### *Tests of Heterogeneity of Crosses With Junior*

In studies such as the present it is possible that the varieties used as parents are not genetically pure, and that the plants selected as parents from the particular variety are not of the same genotype. To determine whether or not this may have happened in this study, the separate crosses were tested for heterogeneity on the basis of individual  $F_3$  families, using the method outlined by Goulden (3). The results of these tests are presented in Table 5. In all five crosses the total chi-square and pooled chi-square values indicate that the data fit the assumed ratios. The heterogeneity chi-square values indicate that the families are not heterogeneous. Therefore, it is safe to assume that for any particular variety the plants chosen as parents were of the same genotype.

TABLE 5.—CHI-SQUARE VALUES OBTAINED BY TESTING THE HETEROGENEITY OF  $F_3$  FAMILIES  
( $P > 0.05$  for all chi-square values)

Cross	Assumed ratio	Total		Pooled		Heterogeneity	
		X <sup>2</sup>	D.F.	X <sup>2</sup>	D.F.	X <sup>2</sup>	D.F.
Junior × O.A.C. 21	3:1	7.04	5	0.35	1	6.69	4
Junior × Titan	3:1	5.62	7	0.80	1	4.82	6
Junior × Ogalitsu	13:3	2.45	6	1.51	1	0.94	5
Junior × Anoidium	15:1	4.70	3	0.26	1	4.44	2
Junior × Jet	1:3	4.05	5	0.43	1	3.62	4

TABLE 6.—PERCENTAGE OF INFECTED PLANTS OF PARENTS,  $F_1$ , AND  $F_2$  OF CROSSES INOCULATED WITH RACE 6 OF *U. hordei* AND GROWN IN THE FIELD

Material	Total plants	No. Infected	% Infected
O.A.C. 21 $\times$ Jet $F_1$	5	0	0.0
O.A.C. 21 $\times$ Jet $F_2$	126	3	2.4
Titan $\times$ Jet $F_1$	21	0	0.0
Titan $\times$ Jet $F_2$	318	4	1.2
Ogalitsu $\times$ Jet $F_1$	3	0	0.0
Ogalitsu $\times$ Jet $F_2$	317	12	3.8
Anoidium $\times$ Jet $F_1$	18	0	0.0
Anoidium $\times$ Jet $F_2$	281	2	0.7
Titan $\times$ O.A.C. 21 $F_1$	19	0	0.0
Titan $\times$ O.A.C. 21 $F_2$	413	0	0.0
Ogalitsu $\times$ O.A.C. 21 $F_1$	23	0	0.0
Ogalitsu $\times$ O.A.C. 21 $F_2$	292	0	0.0
O.A.C. 21 $\times$ Anoidium $F_1$	25	0	0.0
O.A.C. 21 $\times$ Anoidium $F_2$	342	0	0.0
Titan $\times$ Ogalitsu $F_1$	6	0	0.0
Titan $\times$ Ogalitsu $F_2$	423	0	0.0
Titan $\times$ Anoidium $F_1$	14	0	0.0
Titan $\times$ Anoidium $F_2$	340	0	0.0
Ogalitsu $\times$ Anoidium $F_1$	21	0	0.0
Ogalitsu $\times$ Anoidium $F_2$	436	0	0.0
O.A.C. 21	365	3	0.8
Titan	456	0	0.0
Ogalitsu	330	0	0.0
Anoidium	334	0	0.0
Jet	247	1	0.4
Junior	228	164	71.9

One of the five families of the cross Junior  $\times$  O.A.C. 21 did not fit the assumed 3:1 ratio (chi-square = 4.73 and  $P < 0.05$ ). However, the data from this family were included in the results since the deviation was probably due to chance. The ratios for all of the other  $F_3$  families in all crosses did fit the assumed ratios.

*Inheritance of Reaction to Covered Smut in Crosses Between Resistant Parents*

The reactions of parents,  $F_1$ , and  $F_2$  of crosses between resistant varieties are presented in Table 6. The distributions of parental rows,  $F_2$  rows, and  $F_3$  progenies are presented in Table 7.

It will be noted that the  $F_1$ ,  $F_2$ , and  $F_3$  generations of the crosses among the four varieties O.A.C. 21, Titan, Ogalitsu, and Anoidium were all resistant. Although two of the  $F_3$  progeny rows of the cross Titan  $\times$  O.A.C. 21 fell in the 1 to 5 per cent infection class, these must be regarded as resistant since three of the O.A.C. 21 checks also appeared in this class.

## Material

[illegible]

Apparently these varieties all have one gene for resistance in common, or have different genes that are very closely linked. Segregation took place, however, among the progeny of crosses between the resistant variety Jet and the other four resistant varieties.

The results from these crosses were of little value for checking the interpretations of the data obtained from crosses involving Junior. The low soil temperatures that occurred during the early stages of growth probably resulted in a rather high proportion of escapes from infection by plants with susceptible genotypes. This could account for the unexpectedly high proportion of  $F_2$  rows and  $F_3$  lines containing few or no smutted plants. Furthermore, in view of the differential response to temperature among varieties noted by Tapke (13), and since Junior did not occur in any of the crosses, the reaction of Junior could not be used to distinguish segregating from susceptible  $F_3$  lines. Therefore, the lines could not be classified according to the genotypes of the  $F_2$  plants.

#### *Association of Characters*

Junior differed from each of the resistant parents in one or more morphological characters already assigned to a linkage group. According to several investigators most of the characters are governed by single genes and are independently inherited. Linkage studies in barley have been summarized by Robertson and co-workers (7, 8, 9) and by Smith (11). In this study an attempt was made to verify previous reports as to the mode of inheritance of these characters, as well as to check their relationship with one another and with the genes conditioning reaction to covered smut. Appropriate chi-square tests were made to test goodness of fit for the individual characters, and independence where two characters were considered together. The chi-square values obtained are summarized in Table 8.

Eight different morphological characters were studied, each occurring in one or more of the crosses. The character non-6-rowed vs. 6-rowed is a marker for linkage group I. Toothed vs. untoothed lemma also occurs in this group. The marker for group II is black vs. white lemma and pericarp. Hulled vs. naked seed is a marker for group III. This group also contains a gene governing awn barbing and a complementary factor for blue aleurone. In group V the gene for rough vs. smooth awns is known to be associated with genes for long vs. short-haired rachilla and pubescent vs. non-pubescent rachis. The parents did not differ by any genes known to be in the remaining three linkage groups.

Table 8 shows that in most crosses inheritance of these characters is governed by a single gene. However, in two of the three crosses where awn barbing was studied, two genes are apparently involved. In the other cross the parents differ by a single gene pair. Rachis pubescence also appears to be conditioned by two genes.

In the test of independence of row number and smut reaction in the cross Junior  $\times$  Jet, the chi-square value was significant ( $P < 0.05$ ). However, when the data were grouped according to genotype of the  $F_2$  plants, the chi-square test showed that the characters are independent. Similarly, the apparent association between lemma teeth and smut reaction

TABLE 8.—SUMMARY OF CHI-SQUARE TESTS FOR MODE OF INHERITANCE AND INDEPENDENCE OF CHARACTERS IN CROSSES WITH JUNIOR

Characters and crosses	N	Assumption	X <sup>2</sup>	D.F.
<i>Junior</i> × <i>O.A.C. 21</i>				
Hulled vs. naked (Nn)	163	3:1	0.73	1
Long vs. short-haired rachilla (Ss)	163	3:1	3.48	1
Blue vs. non-blue aleurone (Bl bl)	162	3:1	2.37	1
Hull adherence, rachilla hairs	163	Indep.	0.02	1
Hull adherence, aleurone colour	162	Indep.	63.32**	1
Rachilla hairs, aleurone colour	162	Indep.	0.72	1
Smut reaction, hull adherence	163	Indep.	14.40	10
Smut reaction, rachilla hairs	163	Indep.	5.85	10
Smut reaction, aleurone colour	162	Indep.	13.34	10
<i>Junior</i> × <i>Titan</i>				
Rough vs. smooth awns (Rr)	186	13:3	0.03	1
Hulled vs. naked (Nn)	186	3:1	0.01	1
Toothed vs. untoothed lemma (Gg)	186	3:1	1.21	1
Pubescent vs. non-pubescent rachis (Hr hr)	186	15:1	0.52	1
Awns, hull adherence	186	Indep.	0.48	1
Awns, lemma teeth	186	Indep.	4.98*	1
Awns, rachis pubescence	186	Indep.	67.68**	1
Hull adherence, lemma teeth	186	Indep.	2.69	1
Hull adherence, rachis pubescence	186	Indep.	0.97	1
Lemma teeth, rachis pubescence	186	Indep.	16.07**	1
Smut reaction, awns	186	Indep.	5.75	9
Smut reaction, hull adherence	186	Indep.	6.72	9
Smut reaction, lemma teeth	186	Indep.	18.67*	9
Smut reaction, rachis pubescence	186	Indep.	9.05	9
<i>Junior</i> × <i>Ogalitsu</i>				
Rough vs. smooth awns (Rr)	190	3:1	0.01	1
Hulled vs. naked (Nn)	190	3:1	0.07	1
Long vs. short-haired rachilla (Ss)	94	3:1	0.13	1
Awns, hull adherence	190	Indep.	1.96	1
Awns, rachilla hairs	94	Indep.	32.04**	1
Hull adherence, rachilla hairs	94	Indep.	5.28*	1
Smut reaction, awns	190	Indep.	9.03	7
Smut reaction, hull adherence	190	Indep.	6.45	7
Smut reaction, rachilla hairs	94	Indep.	3.48	6
<i>Junior</i> × <i>Anoidium</i>				
Rough vs. smooth awns (Rr)	167	13:3	5.94*	1
Hulled vs. naked (Nn)	167	3:1	2.44	1
Toothed vs. untoothed lemma (Gg)	161	3:1	1.51	1
Long vs. short-haired rachilla (Ss)	167	3:1	1.62	1
Blue vs. non-blue aleurone (Bl bl)	167	3:1	2.44	1
Awns, hull adherence	167	Indep.	0.21	1
Awns, lemma teeth	167	Indep.	13.77**	1
Awns, rachilla hairs	167	Indep.	13.77**	1
Awns, aleurone colour	167	Indep.	0.02	1
Hull adherence, lemma teeth	161	Indep.	3.64	1
Hull adherence, rachilla hairs	167	Indep.	0.12	1
Hull adherence, aleurone colour	167	Indep.	64.68**	1
Lemma teeth, rachilla hairs	161	Indep.	0.02	1
Lemma teeth, aleurone colour	161	Indep.	4.56*	1
Rachilla hairs, aleurone colour	167	Indep.	0.12	1
Smut reaction, awns	167	Indep.	0.13	6
Smut reaction, hull adherence	167	Indep.	4.47	6
Smut reaction, lemma teeth	161	Indep.	2.85	6
Smut reaction, rachilla hairs	167	Indep.	9.55	6
Smut reaction, aleurone colour	167	Indep.	9.39	6
<i>Junior</i> × <i>Jet</i>				
Non-six-rowed vs. six rowed (Vv)	154	3:1	0.42	1
Black vs. white lemma and pericarp (Bb)	154	3:1	0.42	1
Long vs. short-haired rachilla (Ss)	154	3:1	0.06	1
Row number, colour	154	Indep.	3.05	1
Row number, rachilla hairs	154	Indep.	3.72	1
Colour, rachilla hairs	154	Indep.	0.02	1
Smut reaction, row number	154	Indep.	18.83*	8
Smut reaction, colour	154	Indep.	11.04	8
Smut reaction, rachilla hairs	154	Indep.	8.13	8

\* P &lt; 0.05

\*\* P &lt; 0.01

in the cross *Junior* × *Titan* could not be verified when the data were put on a genic basis.

Hull adherence was associated with aleurone colour in the two crosses in which both characters could be studied. Recombination percentages,

calculated by the maximum likelihood method (4), were  $15.78 \pm 3.17$  for Junior  $\times$  O.A.C. 21, and  $14.06 \pm 3.20$  for Junior  $\times$  Anoidium. Evidently the gene governing aleurone colour in these two crosses is the one designated *Bl2*, in Group III. The significant chi-square value obtained for the test of independence of hull adherence and length of rachilla hairs in the cross Junior  $\times$  Ogalitsu was undoubtedly due to chance. No other evidence was obtained that would indicate such a relationship, and it has never been reported.

Rachilla hair length was associated with awn barbing in two crosses, as would be expected from previously published results. In the cross Junior  $\times$  Ogalitsu a recombination percentage of  $18.24 \pm 4.49$  was obtained. In the cross Junior  $\times$  Anoidium no recombination value was calculated. Apparently two genes were involved in the inheritance of awn barbing in this cross. Since  $F_3$  plants were not classified for awn barbing, the genotype of the  $F_2$  plants could not be deduced, and linkage intensity could not be calculated. Awn barbing was also associated with rachis pubescence in the cross Junior  $\times$  Titan. Again, a linkage value could not be calculated, since neither character was simply inherited. It appears that one of the genes governing rachis pubescence in this cross is the gene *Hr2*, known to be in group V. There were no segregates with rough awns and non-pubescent rachises. This would be expected since previous workers have found the gene *Hr2* to be completely linked with the gene *R* for rough awns in group V.

Awn barbing was associated with lemma teeth in the two crosses where Titan and Anoidium were the resistant parents, and lemma teeth with rachis pubescence in the cross Junior  $\times$  Titan. Again, no linkage intensities could be calculated.

#### DISCUSSION

An interesting feature of the data obtained from crosses between Junior and resistant varieties is that, in every cross, the distributions of the  $F_2$  rows and  $F_3$  lines were skewed toward the resistant classes. Part of this can be attributed to escapes from infection, and, in four of the crosses, to dominance. However, these two factors cannot account for all of the skewness.

The negative correlations between stand and infection of the  $F_3$  lines of each of the crosses indicate that distributions were also affected by mortality of infected seedlings. Variations in the values obtained for "r" suggest that the progeny of some crosses were influenced more than the progeny of others. This could be the result of environmental conditions, or of hereditary differences among the parental varieties. Unfortunately, it was not possible to calculate the effect of seedling mortality on the individual lines.

Loss of susceptible plants tended to complicate interpretation of the results. In the crosses where resistance was dominant, susceptible classes would not be expected to be appreciably disturbed by seedling mortality. On the other hand, in the cross in which resistance was recessive, it is the resistant class that would not be expected to be disturbed by seedling mortality. Therefore, when the  $F_3$  lines in each cross were grouped into



two classes only, the effect of seedling mortality should have been virtually eliminated. The total and pooled chi-square values in Table 5 show that the observed ratios all fit the expected. Thus it may be concluded that the resistance of each of the varieties O.A.C. 21 and Titan is governed by a single dominant gene, that of Ogalitsu by a dominant and a recessive, that of Anoidium by two dominant, and that of Jet by a single recessive.

No segregation occurred among the progeny of the crosses between the resistant parents O.A.C. 21, Titan, Ogalitsu, and Anoidium. The genes for resistance carried by the first two varieties and one of the genes for resistance of the others must be allelic, or very closely linked. In crosses between Jet and the other resistant parents segregation for smut reaction occurred. The gene governing the resistance of Jet, therefore, is obviously different from any of the others.

Data from crosses grown in the greenhouse were much more reliable than data from crosses grown in the field. The difference illustrates the importance of controlled temperatures in studies of the inheritance of reaction to covered smut of barley. Unfavourable environmental conditions no doubt account for the failure of most other workers to obtain results suited to genic analysis.

Robertson *et al.* (7) suggested the symbol *Uh uh* for the character resistance vs. susceptibility to *U. hordei*. This has been used by Shands (10) to designate the gene carried by Brachytic. *Uh* could be the gene which appears to be common to Titan, O.A.C. 21, Ogalitsu, and Anoidium; or, it could be the second dominant gene of Anoidium. In any case, Anoidium carries a dominant gene not previously reported, which is designated *Uh2*. The recessive gene carried by Ogalitsu is tentatively designed *uh3* and the gene carried by Jet, *uh4*.

The associations between characters reported here agree fairly well with previous findings. The gene governing aleurone colour in the crosses involving O.A.C. 21 and Anoidium is undoubtedly the gene B12 located in group III, since an association was found with hull adherence in each cross. The gene *R* for rough awns, located in group V, was apparently involved in crosses between Junior and Ogalitsu, Titan, and Anoidium. Where studied, the expected association was found between awn barbing and rachilla hairs. However, Junior apparently differs from Titan and Anoidium by two genes for awn barbing. The location of the other gene is in question, but is probably in group I, since it was associated with lemma teeth in both crosses. No genes for awn barbing have been assigned to group I. However, two are known which have not been assigned to any linkage group (11). The apparent association between lemma teeth and aleurone colour in the cross with Anoidium was probably due to chance.

Evidently one of the genes governing rachis pubescence is the gene *Hr2* in group V. Another, *Hr*, is located in group I. Since rachis pubescence also was associated with lemma teeth in the cross with Titan, it is most probable that this is the gene involved here. This supports the idea that the second gene for awn barbing discussed above is also in group I.

No association was found between the two dominant genes for resistance and genes governing the inheritance of lemma teeth, hull adherence, aleurone colour, awn barbing, and length of rachilla hairs; and between

the gene carried by Titan and the gene governing inheritance of rachis pubescence. There was no association between *uh3* and genes for hull adherence, awn barbing, and length of rachilla hairs; or between *uh4* and genes for number of kernel rows, lemma and pericarp colour, and length of rachilla hairs.

It is encouraging that each of the varieties Ogalitsu, Anoidium, and Jet, carries a different gene for resistance. One or more of these can be combined with the gene carried by Titan. At the present time information is not complete with regard to the interaction between these genes for resistance and the physiologic races of *U. hordei*. Therefore, it is not possible as yet to determine the best combination of genes for maximum resistance to known races.

#### ACKNOWLEDGEMENTS

The writer expresses his thanks to L. P. V. Johnson, Professor of Genetics and Plant Breeding, University of Alberta, for helpful suggestions and criticisms; to V. F. Tapke, United States Department of Agriculture, for seed of the differential hosts; and to W. J. Cherewick, Plant Pathology Laboratory, Winnipeg, for inoculum of race 6 of the pathogen. The writer also expresses his appreciation for assistance in carrying out this study to members of the staff of the Cereal Breeding Laboratory, Experimental Farm, Lethbridge.

#### REFERENCES

1. Briggs, F. N. Dehulling barley seed with sulphuric acid to induce infection with covered smut. *J. Agr. Research* 35:907-914. 1927.
2. Faris, J. A. Physiologic specialization of *Ustilago hordei*. *Phytopathology* 14:537-557. 1924.
3. Goulden, C. H. Methods of statistical analysis. 2nd ed. John Wiley and Sons, Inc., New York, N.Y. 1952.
4. Immer, F. R. Calculating linkage intensities from  $F_2$  data. *Genetics* 19:119-136. 1934.
5. Johnston, W. H. Studies on the dehulling of barley kernels with sulphuric acid and on the inheritance of reaction to covered smut *Ustilago hordei* (Pers.) K. and S. infection in crosses between Glabron and Trebi barleys. *Can. J. Research* 11:458-473. 1934.
6. Pugsley, A. T., and A. Vines. Breeding Australian varieties resistant to covered smut. *J. Australian Inst. Agr. Sci.* 12:44-47. 1946.
7. Robertson, D. W., G. A. Wiebe, and F. R. Immer. A summary of linkage studies in barley. *J. Amer. Soc. Agron.* 33:47-64. 1941.
8. Robertson, D. W., G. A. Wiebe, and R. G. Shands. A summary of linkage studies in barley: Supplement I, 1940-46. *J. Amer. Soc. Agron.* 39:464-473. 1947.
9. Robertson, D. W., G. A. Wiebe, and R. G. Shands. A summary of linkage studies in barley: Supplement II, 1947-1953. *Agron. J.* 47:418-425. 1955.
10. Shands, R. G. Inheritance of covered smut resistance in two barley crosses. *Agron. J.* 48:81-86. 1956.
11. Smith, L. Cytology and genetics of barley. *Botan. Rev.* 17:1-51; 133-202; 285-355. 1951.
12. Tapke, V. F. New physiologic races of *Ustilago hordei*. *Phytopathology* 35:970-976. 1945.
13. Tapke, V. F. Further studies on environment after seedling emergence and other factors that influence the incidence of barley covered smut. *Phytopathology* 42:117-118. 1952.
14. Woodward, R. W., and D. C. Tingy. Inoculation experiments with covered smut of barley. *J. Amer. Soc. Agron.* 33:632-642. 1941.

## RECENT TRIALS WITH NEW ACARICIDES IN BRITISH COLUMBIA ORCHARDS<sup>1</sup>

R. S. DOWNING<sup>2</sup>

Canada Department of Agriculture, Summerland, British Columbia

[Received for publication May 17, 1957]

### ABSTRACT

In British Columbia, the following acaricides gave good control of the European red mite, *Metatetranychus ulmi* (Koch), and the brown mite, *Bryobia arborica* M. & A., when applied to apple trees at the pink bud stage: chlorfensone (*p*-chlorophenyl *p*-chlorobenzene sulphonate), fensone (*p*-chlorophenyl benzene sulphonate), chlorbenside (*p*-chlorobenzyl *p*-chlorophenyl sulphide), Genite 923 (2,4-dichlorophenyl benzene sulphonate), Chlorobenzilate (ethyl 4,4'-dichlorobenzilate), and Kelthane (1,1-bis (chlorophenyl) 2,2,2-trichloroethanol). Trithion [O,O-diethyl S-(*p*-chlorophenylthiomethyl) phosphorodithioate] gave good control of the European red mite; it was not tested in the pre-bloom stage against the brown mite.

A summer spray of Kelthane or Trithion gave good initial and residual control of European red mite and brown mite; Diazinon (O,O-diethyl-O-[2-isopropyl-4-methyl-pyrimidyl (6)] thiophosphate) gave fair initial control but lacked residual effectiveness. Chlorobenzilate gave good control of the brown mite, the only species against which it was tested in the summer.

Chlorfensone and chlorbenside injured apple foliage when applied at the pink bud stage. Chlorfensone, chlorbenside and fensone injured the fruit of some varieties and Genite 923 severely injured apple foliage when used in summer.

### INTRODUCTION

In the fruit growing districts of British Columbia the control of mites has been particularly important in the orchardist's spray program for the last 10 years. Of the several species concerned, the European red mite, *Metatetranychus ulmi* (Koch), which attacks all tree fruits grown in the Province, has consistently been the most troublesome. Most of the work on the control of mites has been directed against this species.

As recently as a decade ago, chief reliance was placed on the use of dormant oil to control the European red mite; the oil is toxic to overwintered eggs. But when the present-day synthetic organic acaricides began to be available, the logic of attacking the mite with a pink bud spray was obvious. This is a report on a considerable amount of experimental work carried out at the Summerland laboratory to compare dormant sprays with sprays applied at the pink bud stage, when most of the mite larvae had hatched, and with summer sprays against mature and immature stages. The effects of some of these acaricides on other species of orchard mites was studied whenever the mites became available in sufficient numbers.

### MATERIALS AND METHODS

The acaricides were formulated as wettable powders unless otherwise noted. The generic or common names of the first three have been officially approved in the United Kingdom; they have not been given common names in North America with the exception of chlorfensone, which is known as "ovex". The preparations were as follows:

<sup>1</sup>Contribution No. 3628, Entomology Division, Science Service, Department of Agriculture, Ottawa, Ont.

<sup>2</sup>Associate Entomologist.

1. Chlorfensone (*p*-chlorophenyl *p*-chlorobenzene sulphonate), 50 per cent; *Ovotran*, Dow Chemical Company, Midland, Mich.
2. Fensone (*p*-chlorophenyl benzene sulphonate), 50 per cent; *Murvesco*, Murphy Chemical Company, Wheathampstead, England.
3. Chlorbenside (*p*-chlorobenzyl *p*-chlorophenyl sulphide), 20 per cent; *Elimite*, Boots Pure Drug Company, Nottingham, England.
4. Genite 923, 50 per cent emulsifiable liquid of 2,4-dichlorophenyl benzene sulphonate; General Chemical Division, Allied Chemical & Dye Corp., New York, N.Y.
5. Chlorobenzilate, 25 per cent ethyl 4,4'-dichlorobenzilate; Geigy Chemical Corp., Bayonne, N.J.
6. Kelthane, 18.5 per cent 1,1-bis(chlorophenyl) 2,2,2-trichloroethanol; Rohm & Haas Co., Philadelphia, Pa.
7. Trithion 4E, an emulsifiable liquid containing 4 lb. of O,O-diethyl S-(*p*-chlorophenylthiomethyl) phosphorodithioate per U.S. gal.; Stauffer Chemical Company, Mountain View, Calif.
8. Diazinon, 25 per cent O,O-diethyl-O-[2-isopropyl-4-methyl-pyrimidyl (6)] thiophosphate; Geigy Chemical Corp., Bayonne, N.J.

Except for one instance, in which a conventional high-volume hand-gun sprayer was used, the acaricides were applied in commercial orchards with a Turbo-Mist concentrate, air-blast sprayer\*. The concentrate sprayer applied approximately 75 gal. of spray liquid per acre, the hand-gun sprayer approximately 1,000 gal.

Unless otherwise stated, estimates of mite populations were made by taking a 20-leaf sample from one quadrant of each of five trees, or 100 leaves per treatment. Leaves infested with the European red mite were processed by the method of Venables and Dennys (8); those infested with the brown mite, *Bryobia arborea* M. & A. by the brush method of Henderson and McBurnie (4) as modified by Morgan *et al.* (7).

## RESULTS AND DISCUSSION

### *Chlorfensone, Fensone, and Chlorbenside*

These three acaricides are somewhat similar chemically and all are effective against eggs and other immature stages of several mites (2, 5). They are considered to have low toxicity to mammals and insects.

Chlorfensone has been under trial at Summerland since 1949. In 1950 it gave excellent control of the European red mite when applied at the pink bud stage of apple trees (3). Table 1 shows that, as a pink spray, it was much more effective in controlling this mite than a dormant spray of oil plus lime-sulphur.

During 1951 and 1952 several acaricidal trials were carried out against the brown mite. Table 2 shows that chlorfensone applied at the pink bud stage of apples, a few days after the eggs hatch, effectively controlled this mite as it did the European red mite.

In 1954, Armstrong *et al.* (1) found fensone and chlorbenside very effective acaricides. At Summerland a pink bud application of either material controlled the European red mite for the remainder of the season, as did chlorfensone. When applied in the summer against immature and adult stages of the European red mite, they were not effective until about 2 weeks after the application, presumably because they do not readily kill adult mites. Another disadvantage in using these materials in British Columbia in summer is that they may injure fruits. Chlorfensone, for

\*Okanagan Turbo Sprayers Ltd., Penticton, B.C.

TABLE 1.—AVERAGE NUMBERS OF THE EUROPEAN RED MITE AFTER APPLICATION OF TWO ACARICIDES BY CONCENTRATE SPRAYER TO DELICIOUS APPLE AT TWO STAGES, SUMMERLAND, B.C., 1954

Stage	Acaricide	Amount per acre	Mites per leaf <sup>1</sup> June 25
Dormant	Lime-sulphur <sup>2</sup>	16.5 gal.	11.3
	Dormant oil <sup>3</sup>	6.75 gal.	
Pink bud	Chlorfensone, 50%	6 lb.	0.2
	Check	no treatment	19.4

<sup>1</sup>Based on 40 leaves from each of 5 trees<sup>2</sup>Specific gravity 1.28; Oliver Chemical Co., Penticton, B.C.<sup>3</sup>Shell Helix 29, viscosity 100° F., 200-220 S.S.U.; Shell Oil Co., Penticton, B.C. Emulsified with soya flour, 0.5 lb. per 100 gal.

TABLE 2.—AVERAGE NUMBERS OF THE BROWN MITE AFTER APPLICATION OF TWO ACARICIDES BY CONCENTRATE SPRAYER TO NEWTOWN APPLE IN THE PINK BUD STAGE, SUMMERLAND, B.C., 1952

Acaricide	Amount per acre	Mites per leaf <sup>1</sup>	
		June 30	Aug. 20
Chlorfensone, 50%	8 lb.	0.2	3.3
Malathion, 25%	12 lb.	0.4	13.3
Check	no treatment	9.0	52.4

<sup>1</sup>Based on 20 leaves from each of 5 trees

example, caused pronounced injury to pear fruitlets and to Winesap apples; fensone injured the lenticels of Newtown apples; chlorbenside caused a "corking" of the lenticels of Delicious apple. When applied in pre-bloom sprays chlorfensone and chlorbenside caused necrotic spotting on apple foliage; fensone, however, was non-injurious.

In 1955, pink bud applications of chlorfensone, fensone, and chlorbenside, when used at either 1 or 2 lb. of toxicant per acre, gave good control of the European red mite (Table 3). Against the brown mite, 2 lb. gave satisfactory control but 1 lb. did not (Table 4). Evidently, effectiveness and phytotoxicity considered, the best time to apply these acaricides to apples in British Columbia is at the pink bud stage; to pears, at the white bud stage.

#### Genite 923

The active ingredient of Genite 923 is a structural isomer of chlorfensone. Like chlorfensone, Genite 923 is comparatively ineffective against adult mites, but it is highly toxic to eggs and other immature stages; and, like chlorfensone, Genite 923 has little toxicity to insects and mammals.

Applied as a summer treatment at Kelowna, B.C., in 1949, Genite 923 severely defoliated Jonathan apple trees. In pre-bloom application, however, Madsen (6) reported that Genite 923 had given good control of the European red mite in California. Genite, therefore, was re-examined as a pink bud spray in 1955. Tables 3 and 4 show that at 1 gal. per acre it gave good control of both the European red mite and the brown mite, with no sign of injury to foliage or fruit.

TABLE 3.—AVERAGE NUMBERS OF THE EUROPEAN RED MITE AFTER APPLICATION OF TEN ACARICIDES BY CONCENTRATE SPRAYER TO DELICIOUS APPLE IN THE PINK BUD STAGE, OLIVER, B.C., 1955

Acaricide	Amount per acre	Mites per leaf <sup>1</sup>	
		June 2	Aug. 2
Fensone, 50%	4 lb.	0.1	1.9
Fensone, 50%	2 lb.	0.1	0.6
Chlorfensone, 50%	4 lb.	0.0	2.5
Chlorfensone, 50%	2 lb.	0.2	0.6
Chlorbenside, 20%	10 lb.	0.1	1.4
Chlorbenside, 20%	5 lb.	0.1	1.8
Chlorobenzilate, 25%	8 lb.	0.1	2.1
Genite EM 923, 50%	1 gal.	0.0	1.8
Kelthane, 18.5%	8 lb.	0.1	1.6
Trithion 4E	1 gal.	0.1	0.8
Check	no treatment	0.3	19.8

<sup>1</sup>Based on 20 leaves from each of 5 trees

TABLE 4.—AVERAGE NUMBERS OF THE BROWN MITE AFTER APPLICATION OF EIGHT ACARICIDES BY CONCENTRATE SPRAYER TO DELICIOUS APPLE IN THE PINK BUD STAGE, CAWSTON, B.C., 1955

Acaricide	Amount per acre	Mites per leaf <sup>1</sup>	
		June 14	Aug. 16
Fensone, 50%	4 lb.	0.2	1.8
Fensone, 50%	2 lb.	2.3	41.6
Chlorfensone, 50%	4 lb.	0.6	5.6
Chlorfensone, 50%	2 lb.	0.2	18.5
Chlorbenside, 20%	10 lb.	0.6	7.6
Chlorbenside, 20%	5 lb.	1.8	21.2
Genite EM 923, 50%	1 gal.	0.9	2.4
Kelthane, 18.5%	8 lb.	0.1	1.8
Check	no treatment	28.0	sprayed

<sup>1</sup>Based on 20 leaves from each of 5 trees

TABLE 5.—AVERAGE NUMBERS OF THE BROWN MITE AFTER APPLICATION OF FOUR ACARICIDES BY HAND-GUN SPRAYER TO DELICIOUS APPLE IN JULY, CAWSTON, B.C., 1955

Acaricide	Amount per 100 gal.	Mites per leaf <sup>1</sup>		
		Before spraying July 1	After spraying	
			July 11	Aug. 17
Kelthane, 18.5%	1 lb.	15.6	3.8	0.1
Trithion, 4E	1 pt	21.6	8.6	0.0
Diazinon, 25%	2 lb.	5.7	1.6	3.9
Chlorobenzilate, 25%	1 lb.	9.6	4.6	0.5
Check	no treatment	22.0	5.6	13.4

<sup>1</sup>Based on 20 leaves from each of 5 trees



*Chlorobenzilate*

Chlorobenzilate is another acaricide with low mammalian and insect toxicities. It gave good control of the European red mite when applied during the pink bud stage to apples at 8 lb. per acre (Table 3). In post-bloom applications chlorobenzilate gave good control of the brown mite (Table 5).

*Kelthane*

One of the most recently developed acaricides, Kelthane, like the others previously mentioned, is reported to have a low toxicity to mammals and insects. Field trials with Kelthane in British Columbia were begun in 1955. Tables 3, 4 and 5 show that, when applied to apple at the pink bud stage against the European red mite or the brown mite, Kelthane gave good control of both species for the entire season. Applied in the summer against the brown mite, it gave good initial and residual mortality of all stages (Table 5). As a wettable powder, Kelthane caused no fruit or foliage injury in any of the applications. The product has one disadvantage that would somewhat limit its use; it is incompatible with lime-sulphur, which, in the fruit-growing areas of British Columbia, is commonly used in pre-bloom sprays as a fungicide. In the pre-bloom period Kelthane would have to be applied as a special spray, a feature that would not appeal to fruit growers.

*Trithion and Diazinon*

As might be expected, the organic phosphates Trithion and Diazinon are toxic to insects as well as mites; in that respect they differ from the preceding preparations, which have very little toxicity to insects.

When applied to apples at the pink bud stage, Trithion gave good control of the European red mite (Table 3). Its long residual effectiveness was not demonstrated, however, until it was used in summer against immature and adult stages of mites. Table 6 shows that, where Trithion was applied against the European red mite, not only did it give good initial control but, even after 3 weeks, there was an average of less than one mite per leaf, whereas there were more than 11 mites per leaf where Diazinon had been used. Mites were so numerous in the check plot that the trees had to be sprayed before the 3-week period had elapsed. As other non-systemic organic phosphates, i.e., Diazinon, TEPP, parathion, and mala-

TABLE 6.—AVERAGE NUMBERS OF THE EUROPEAN RED MITE AFTER APPLICATION OF TWO ACARICIDES BY CONCENTRATE SPRAYER TO WINESAP APPLE IN JULY, OLIVER, B.C., 1955

Acaricide	Amount per acre	Mites per leaf*	
		July 29	Aug. 12
Trithion, 4E	1 gal.	1.7	0.5
Diazinon, 25%	16 gal.	5.5	11.4
Check	no treatment	20.1	sprayed

\*Average of two replications per treatment; 25 leaves sampled from each of 4 trees for each replicate

thion, are characteristically weak in residual action, the persistence of Trithion is an encouraging development. Results with the compound against the brown mite were somewhat similar to those against the European red mite (Table 5).

For growers who might wish to use a general insecticide and acaricide, Trithion and Diazinon should have special interest. These compounds might be used in a pre-bloom application to control various leaf rollers, the eye-spotted bud moth, *Spilonota ocellana* (D. & S.), aphids and mites and in post-bloom applications to control aphids, scale insects, mites, and the codling moth, *Carpocapsa pomonella* (L.).

#### ACKNOWLEDGEMENTS

Thanks are due to F. Seemungal, formerly of the Entomology Laboratory, Summerland, and to G. D. Halvorsen, for their assistance in the application of sprays and in the estimation of mite populations.

#### REFERENCES

1. Armstrong, T., G. G. Dustan, and R. S. Downing. A comparative study of three acaricides. *Ann. Rept. Entomol. Soc. Ontario* 85 (1954): 5-17. 1955.
2. Cranham, J. E., D. J. Higgons, and H. A. Stevenson. *p*-chlorobenzyl *p*-chlorophenyl sulphide: A new ovicide for control of red spider. *Chem. & Ind.*, pp. 1206-1207. 1953.
3. Downing, R. S. Acaricide trials in British Columbia orchards, 1950. *Proc. Entomol. Soc. Brit. Columbia* 47:1-4. 1951.
4. Henderson, C. F., and H. Y. McBurnie. Sampling technique for determining populations of citrus red mite and its predators. *U.S. Dept. Agr. Circ.* 671. 1943.
5. Kirby, A. H. M., and R. P. Tew. Agricultural acaricides. *Rept. Progress Appl. Chem.* 37:263-276. 1952.
6. Madsen, H. F. Early sprays for mite control. *Calif. Agr.* 9(2):4, 15. 1955.
7. Morgan, C. V. G., D. A. Chant, N. H. Anderson, and G. L. Ayre. Methods for estimating orchard mite populations, especially with the mite brushing machine. *Can. Entomologist* 87:189-200. 1955.
8. Venables, E. P., and A. A. Dennys. A new method of counting orchard mites. *J. Econ. Entomol.* 34:324. 1941.

# POLYEMBRYONY IN TOMATOES<sup>1</sup>

H. H. MARSHALL

*Canada Department of Agriculture, Brandon, Manitoba*

[Received for publication May 21, 1957]

## ABSTRACT

Twenty-nine polyembryonic seeds were found among 26,000 tomato seeds. These gave rise to twin seedlings. Five of the polyembryonic seeds appeared as a "double" seed with two units joined at adjacent sides to form the seed. Five pairs of conjoined seedlings were found. In some pairs, one member was four or five times larger than its mate. All the mature twins appeared to be diploid. One true-breeding mature twin may have been a haploid whose chromosome complement was spontaneously doubled. Most of the twin seedlings were from parents which were heterozygous for the gene *U* (uniform green fruits). The *U*-genotypes of 32 such twins were determined from  $F_2$  segregation. The results indicate that most twins resulted from normal segregation and recombination. Therefore, simple polyembryony appears to be the most likely origin of most tomato twin seedlings. False polyembryony due to fusion of two ovules, and euploid polyembryony may account for a few twins. Sporophytic and cleavage polyembryony appear to be relatively unimportant.

## INTRODUCTION

Polyembryony, or the presence of more than one embryo in a seed, occurs sporadically in many plant genera. The present study attempts, by genetic analysis, to elucidate the mechanisms of polyembryony in the tomato.

## MATERIALS AND METHODS

In 1949, 1950 and 1951, tomato seeds were germinated on paper towels in lots of approximately 1000 seeds, calculated by weight. After the radicles emerged the seeds were examined one at a time for multiple embryos. No seed with multiple embryos produced more than two seedlings.

Twins originated from three sources.  $T_2$ , a conjoined pair, which yielded no further information, was found among seedlings of the variety Quebec No. 5.  $T_1$ ,  $T_3$ ,  $T_4$  and  $T_5$  were obtained from seed harvested from the  $F_1$  hybrid Bounty  $\times$  F.N. 21 while the largest group were from a similar  $F_1$  hybrid Bounty  $\times$  F.N. 23.

Both Bounty and F.N. 21 bear uniform green fruits a character determined by the recessive gene *uu* (5) so the four pairs from these parents should have had uniform green fruits. F.N. 23, like F.N. 21, is a selection of the variety Farthest North, but it has fruit with the dominant dark green base (*U*). Therefore, the latter group of twins belong to an  $F_2$  generation which was segregating for a marker gene. The *U*-genotype of each twin thus supplied information on the genetic relations of a twin to its mate and of both twins to the female parent. Hence, it provided a valuable tool for understanding the process of polyembryony. The *U*-genotype of each surviving twin seedling was determined from  $F_2$  segregation.

<sup>1</sup>Contribution No. 902, Horticulture Division, Experimental Farms Service.

## RESULTS

Twenty-nine polyembryonic seeds were found among 26,000 seedlings. This is approximately one pair of twins in 900 seeds, and a much higher frequency than the four pairs in 18,672 tomato seeds previously reported by Rick (3).

*Types of Twins*

In most pairs, one seedling was larger than the other. In the tables, the larger twin is designated "A" and the smaller "B". In some cases one twin was four or five times larger than its mate. These pairs are designated "dissimilar". Casualties among twins, which differed greatly in size, were 42 per cent, compared with 37 per cent for all twin seedlings.

Five seeds that gave rise to twin seedlings showed some external indication of the presence of two embryos. They appeared as two seeds more or less united by their adjacent flat sides. These are designated "double" in the tables. All other twins developed from what appeared to be normal single seeds.

In five pairs, the seedlings were conjoined along one side for the full length of the hypocotyl. One pair and one individual survived and are designated "conjoined". In all other twin pairs, the hypocotyls were free.

*Twins from Bounty  $\times$  F.N. 21.*

Only four twin seedlings from this source were raised to maturity. Since both parents were homozygous recessive (*uu*) for uniform green, all the twins should have been uniform green. Three seedlings bred as expected (Table 1), but T1B bred true for the dominant green base. In addition, the progeny of T1B showed no segregation for other characters in either  $F_3$  or  $F_4$  from mixed seed of the preceding generation.

The fact that this seedling differed so widely from the expected seems to raise doubt as to its true origin. Mutation or stray pollen from F.N. 23 might account for the green base but not for the lack of segregation. No segregation would be expected from an admixture of a fixed variety, but T1B bears inferior quality fruit unlike any variety grown at the Brandon Farm. Chance alone could produce a true breeding seedling in the  $F_2$  but as the parents differed by several factors other than in colour of fruit the chance is remote. Another possibility is by doubling of the chromosomes in a haploid.

TABLE 1.—FREQUENCY OF *U*-PHENOTYPES IN THE PROGENY OF TOMATO TWINS  $F_2$  FROM BOUNTY  $\times$  F.N. 21

Twin pair No.	A-twins				B-twins				Remarks
	Green base		Uniform		Green base		Uniform		
	1950	1952	1950	1952	1950	1952	1950	1952	
T1	—	—	—	—	10	14	0	0	Conjoined Dissimilar
T3	0	0	10	13	—	—	—	—	
T4	0	0	10	14	0	0	10	14	

*Twins from Bounty × F.N. 23*

Since segregation for uniform green vs. dark green base fruit was expected, the *U*-genotypes of 32 twins from this source was determined from  $F_2$  populations in 1950 and 1952 (Table 2). Nine twins were homozygous for dark green base, 16 were heterozygous, and 7 were homozygous for uniform green. These proportions are very close to the 1:2:1 ratio expected from random segregation and recombination.

The 16 heterozygous plants gave a total of 189 plants with green base fruit and 68 plants with uniform green fruit. This is almost exactly a 3:1 ratio and supports the reliability of the genetic data.

## DISCUSSION

Several types of polyembryony have been found in plants and the discussion will attempt to show which of these types apply to tomatoes.

*False Polyembryony*

False polyembryony (6,1) occurs when the several embryos of a seed are derived from more than one nucleus. It includes multiple seeds formed when more than one ovule develops in a normally one-ovulate seed-like structure. This cannot occur in tomato where the seed coat represents the outer tissue of the ovule.

However, false polyembryony also includes multiple seeds which arise by fusion of separate ovules. The "double" tomato seeds may arise in this way, but without embryological studies it cannot be proved. Twins of this type should differ genetically by chance, and the members of the three surviving pairs from "double" seeds (T19, T21 and T23) do differ genetically (Table 2).

TABLE 2.—FREQUENCY OF *U*-PHENOTYPES IN THE PROGENY OF TOMATO TWINS  $F_2$  FROM BOUNTY × F.N. 23

Twin pair No.	A-twins				B-twins				Remarks
	Green base		Uniform		Green base		Uniform		
	1950	1952	1950	1952	1950	1952	1950	1952	
T6	10	14	0	0	10	15	0	0	Dissimilar
T7	9	10	1	4	9	7	1	4	Dissimilar
T8	7	7	3	6	10	6	0	3	Dissimilar
T9	8	8	2	4	9	7	1	4	Dissimilar
T12	—	9	—	3	—	0	—	14	
T13	—	0	—	13	—	0	—	13	
T15	—	9	—	3	—	9	—	4	Dissimilar
T16	—	10	—	0	—	11	—	0	
T18	—	8	—	5	—	—	—	—	Double
T19	—	13	—	0	—	0	—	13	Double
T20	—	9	—	3	—	9	—	4	Dissimilar
T21	—	13	—	0	—	12	—	3	Double
T23	—	0	—	14	—	14	—	0	Double Dissimilar
T24	—	7	—	5	—	0	—	15	
T26	—	11	—	3	—	13	—	0	Conjoined
T27	—	9	—	2	—	15	—	0	
T28	—	0	—	13	—	—	—	—	

### *Sporophytic Polyembryony*

This type of polyembryony involves apomixis. One or more "buds" of the parent plant develop into embryos which may or may not replace the normal embryos (6). Seedlings of this type have the same genotype as the parent plant so that those from the  $F_1$  hybrid Bounty  $\times$  F.N. 23 should be heterozygous  $Uu$ .

The proportion of segregating types (Table 2) suggests the expected 1:2:1 ratio, and the data do not show a surplus of heterozygous individuals. Therefore, sporophytic polyembryony is unlikely. In five pairs, both twins were homozygous so that neither could have originated in this way.

### *Simple Polyembryony*

Strictly, this type of polyembryony occurs when two or more egg cells are produced in one embryo sac (6). This may occur when the normal number of nuclear divisions leads to the formation of the embryo sac, but one or more nuclei, which normally becomes synergids or antipodals, develop into eggs. However, the same result is obtained when a synergid or antipodal nucleus functions as an egg so both processes will be called simple polyembryony. Twins of both types differ genetically in the same way as normal seedlings.

If seedlings are selected in pairs from  $F_2$  population which is segregating in a 1:2:1 ratio, six combinations of the three genotypes are expected in the ratio given in Table 3. The six classes among the twins of  $F_2$  plants from Bounty  $\times$  F.N. 23 were observed at approximately the calculated proportions. Thus the data fit to a degree the expectations from simple polyembryony with the normal number of nuclear divisions in the embryo sac.

It would seem possible for an egg cell to divide mitotically before fertilization, to give two egg cells. Twin pairs formed by this process would have identical maternal inheritance but the genes received from the pollen would differ at random. The proportions of observed pairs differ from the expected calculated on this basis (Table 3). The group homozygous, but for opposing alleles, would not be possible. Both of these pairs, however, originated from "double" seeds and possibly should be excluded from consideration here. If so, the data appear to support like maternal inheritance. However, a much larger sample would be required to prove the absence of pairs homozygous for opposing alleles.

TABLE 3.—OBSERVED COMBINATIONS OF TWIN PAIRS FROM BOUNTY  $\times$  F.N. 23 COMPARED WITH THEORETICAL RATIOS OBTAINED FROM RANDOM PATERNAL AND MATERNAL INHERITANCE AND FROM RANDOM PATERNAL BUT LIKE MATERNAL INHERITANCE

Combinations possible	Pairs observed	Theoretical ratios	
		Random maternal	Like maternal
Both $UU$	2	1	2
Both $uu$	1	1	2
Both $Uu$	5	4	4
$UU$ and $uu$	2	2	0
$UU$ and $Uu$	3	4	4
$Uu$ and $uu$	2	4	4
Totals	15	16	16



*Cleavage Polyembryony*

The multiple embryos of this type originate by division on an embryo (6). Therefore, the seedlings are genetically identical. On the basis of external morphology, the "conjoined" twins might be expected to be of this type. However, members of the one surviving conjoined pair (T26) differed genetically and so must be the result of fusion or natural grafting. In contrast, conjoined asparagus twins proved identical for the characters studied (2).

Considering the 15 twin pairs of Bounty  $\times$  F.N. 23, 7 pairs differed genetically (Table 3), and so could not be the result of cleavage polyembryony. By chance, some pairs are expected to be identical for *U*-genotype, but 2 more pairs of this type than expected were found. Because of the small sample, this need not be significant; so it is concluded that few if any of the tomato twins resulted from cleavage polyembryony.

*Euploid Polyembryony*

Euploid polyembryony results from various cytological irregularities and may give rise to haploids, triploids, tetraploids, and trisomics (6). In 4 pairs of tomato twins, Rick (3) found 7 diploids and 1 haploid.

All the tomato twin seedlings that reached maturity appeared to be normal diploid plants and all produced seed freely which would not be the case with the above chromosome types (3, 4). Pollen of the surviving twins up to T9B was examined microscopically and appeared normal. Therefore, it is probable that all the mature twins were diploid.

It would seem possible for a haploid to have its chromosome complement doubled spontaneously at an early stage, thus producing a true-breeding diploid. Rick and Butler (5) believe that seldom, if ever, does this occur naturally in tomato shoots, although it does happen in roots. However, it may have happened in T1B, the smaller member of a "dis-similar" pair which bred true. The unexpected genotype of T1B may have been the result of mutation, or an admixture of seed from another source. In either case, however, it is difficult to account for the true-breeding genotype of T1B, unless it were a doubled haploid. All seed used was produced at the Brandon station and T1B was unlike any variety known to the author. Breeding lines in use during the test period would have been genetically similar to Bounty  $\times$  F.N. 21. Since only one possible haploid was obtained from 26,000 seeds, it does not appear that examination of twin seedlings is a practical method for obtaining haploids or true breeding lines. Haploids are generally reduced in vigour and may have been lost among the 37 per cent casualties, most of which occurred while the seedlings were emerging from the seed coat.

**ACKNOWLEDGEMENTS**

The author wishes to acknowledge help and encouragement from the late Erdman Braun and from J. D. Truscott, of the University of Manitoba. He also is indebted to J. M. Rick, of the University of California, for reading an earlier manuscript and offering suggestions on its improvement; and to D. R. Sampson, Horticulture Division, Central Experimental Farm, Ottawa, for his aid in interpreting the data and increasing the clarity of the presentation.

## REFERENCES

1. Gustafsson, A. Apomixis in higher plants. I. The mechanism of apomixis. Lunds Univ. Arsskr. N.F. Avd. 2, 42 (3):1-67. 1946.
2. Randall, T. E., and C. M. Rick. A cytogenetic study of polyembryony in *Asparagus officinalis* L. Amer. J. Botany 32:560-569. 1945.
3. Rick, C. M. A survey of cytogenetic unfruitfulness in the tomato. Genetics 30: 347-362. 1945.
4. Rick, C. M., and W. D. Barton. Cytological and genetical identification of the primary trisomics of the tomato. Genetics 39:640-666. 1954.
5. Rick, C. M., and L. Butler. Cytogenetics of the tomato. Advances in Genetics 7:267-382. 1956.
6. Webber, J. M. Polyembryony. Botan. Rev. 6:575-598. 1940.

# CHEMICAL COMPOSITION OF VARIOUS COMMERCIAL GRADES OF CANADIAN FLUE-CURED TOBACCO<sup>1</sup>

J. M. ELLIOT AND E. C. BIRCH

Canada Department of Agriculture, Delhi, Ontario

[Received for publication February 22, 1957]

## ABSTRACT

A study was made of the chemical composition of 21 commercial grades of Canadian flue-cured tobacco, selected from a 50-acre crop of Hicks variety in 1955. Arbitrary prices were assigned to the various grades of tobacco. Correlation coefficients between the chemical values and the assigned grade prices were calculated. Ethanol extracts, total sugars, reducing sugars, and hygroscopicity gave significant positive correlations; total nitrogen, protein nitrogen, total alkaloids, nicotine, calcium, and magnesium gave negative correlations. These coefficients indicated that quality measured by these laboratory methods conformed with leaf-graded quality. Correlation coefficients were not significant between grade quality and petroleum ether extract, sucrose, starch, ash, silica, potassium, phosphorus, chlorine, sulphur, burn, or pH.

## INTRODUCTION

The chemical composition of commercial grades of flue-cured tobacco has been studied extensively. Darkis and Hackney (7) found that, relative to other grades of flue-cured tobacco and tobacco used in blended cigarettes, the best grades were fairly high in soluble sugars, low to medium in nitrogenous and acid constituents, and medium in nicotine content. Darkis *et al.* (5) showed that nitrogenous constituents and nicotine were most abundant in the upper leaves and least abundant in the middle and lower leaves. The soluble sugars were highest in the middle leaves and decreased in content in the leaves toward both the base and the top of the stalk. As measured by pH, the more acid tobaccos were produced near the top of the stalk. The soluble ash was at a minimum in the middle leaves, increasing in leaves near the ends of the stalk, being a maximum in the leaves at the base of the stalk.

Ward (19) analysed five grades of Canadian flue-cured tobacco and six grades of American flue-cured tobacco and found that, in general, the percentage of soluble sugars was directly proportional to quality. Blick (3) and Askew *et al.* (1) showed that there was a relationship between the ratio of total sugars to total nitrogen and quality. Mason and Lea (14) stated that a correct balance of the chemical constituents must be realized to obtain a satisfactory smoke and that an excess of sugar is undesirable as the smoke is given an acidic characteristic.

Phillips and Bacot (16) showed that grades of flue-cured tobacco which differ in physical properties as judged by "feel" and appearance also differ decidedly in chemical composition. Alcohol extractives, total reducing substances, total sugars, reducing sugars, and starch showed a direct relationship with quality. Total nitrogen, proteins, total pectic substances, pentosans, cellulose, lignin, and oxalic and citric acids showed an inverse relationship with quality. Petroleum ether extractives, ether extractives, methoxyl substances, polyphenols, tannins, *l*-malic acid, and resins and waxes were not apparently related to quality.

<sup>1</sup>Contribution from the Tobacco Division, Experimental Farms Service.

This investigation was conducted to determine the correlations between various leaf constituents and the assigned prices of the subjectively appraised grades of Canadian flue-cured tobacco.

### MATERIALS AND METHODS

Single samples of 21 commercial grades were selected from a 50-acre crop of Hicks variety flue-cured tobacco grown in 1955 on Fox loamy sand under recommended fertilization (9) with supplemental irrigation. Several graders selected a large quantity of each grade of tobacco which was then reselected by additional judges to ensure an accurate representation of the grade.

The grades of flue-cured tobacco are based on stalk position (lugs, cutters, leaf, and tips), colour, texture, body, maturity, elasticity, and aroma. The colour of the grades ranges from lemon-yellow (L) or lightest colour, through orange (O) or medium colour, to dark mahogany (D) or darkest colour. The symbols "G" and "LG" designate green and lemon-green tobaccos, respectively. The individual grades used in this investigation are described in Table 1 in which arbitrary prices for each grade are assigned to show its relative commercial value. This relationship between price and grade will be valid from year to year. The term "quality" is used to signify commercial value or grade-price.

The mid-ribs were removed and the web portions of the leaves were used for analyses. Samples were dried at 95° F. for 48 hours, ground in a Wiley Mill, and stored in air-tight glass jars for analysis. All analyses were made at least in duplicate on each sample.

TABLE 1.—DESIGNATION, DESCRIPTION AND ASSIGNED PRICE OF GRADES

Grades	Description	Price
		¢. per lb.
X2L	Lug fine lemon	61
X4L	Lug fair lemon	47
X50	Lug low orange	32
XND	Lug nondescript	5
CIL	Cutter choice lemon	72
C3L	Cutter good lemon	61
C40	Cutter fair orange	47
C3LG	Cutter good lemon-green	43
C50M	Cutter low orange-mixed	32
BIL	Leaf choice lemon	72
B2L	Leaf fine lemon	63
B3L	Leaf good lemon	55
B40M	Leaf fair orange-mixed	50
B3LG	Leaf good lemon-green	42
B4G	Leaf fair green	27
B6G	Leaf common green	18
BT2L	Leaf-tip fine lemon	63
BT30	Leaf-tip good orange	55
BT40	Leaf-tip fair orange	50
T30	Tip good orange	31
T6D	Tip common dark	18

The percentages of the constituents were calculated on a moisture-free and sand-free basis. Moisture was determined by drying a 2-gram sample over concentrated (above 95 per cent) sulphuric acid at 25° C. for 14 days. These dried samples were then placed in an atmosphere of 58 per cent relative humidity (over a saturated solution of the dihydrate salt of sodium bromide) at 20° C. for 14 days. The increase in weight on a percentage basis was termed hygroscopicity (6). The samples were ashed by charring over a low flame and heating at 500° C. for 2 hours in a muffle furnace. Sand, silica, calcium, and magnesium were determined by the official methods of the A.O.A.C. (2).

The titrimetric method of Harrell (12) was used in the determination of chlorine. Sulphur was determined by the magnesium nitrate method (2). Phosphorus was colorimetrically determined by using ammonium molybdate with stannous chloride reduction. Potassium was measured by means of a Beckman flame spectrophotometer. The pH values were measured with a Beckman Model G pH meter, using 5 gm. of tobacco and 75 ml. of distilled water that had been boiled and cooled to room temperature.

The Perrin method (15) was used for the determination of total nitrogen. This method does not include nitrate nitrogen but laboratory tests have shown that only traces of nitrate nitrogen are found in the cured leaves of flue-cured tobacco. Protein nitrogen was determined by the procedure of Phillips and Bacot (16). Total alkaloid and nicotine content were obtained by the method of Cundiff and Markunas (4).

The Shaffer-Somogyi method as modified by Heinze and Murneek (13) was used for the determination of reducing sugars and sucrose.

The petroleum ether extract was ascertained by the apparatus and method described by Vickery and Meiss (18), using petroleum ether (B.P. 30° to 60° C.). The ethanol extract was obtained by extracting the residual tobacco from the petroleum ether extraction with 95 per cent ethanol for 16 hours. The residue from the ethanol extraction was used for the determination of starch by the diastase method with subsequent acid hydrolysis (2) and the reducing power was determined by the method used for reducing sugars (13).

The burn of tobacco was measured by recording the number of seconds required to burn one inch of cigarette made from ground leaves which had been held in an atmosphere of 58 per cent relative humidity at 20° C. for 7 days.

Coefficients of correlation (17) between the percentages of the constituents and the assigned grade prices have been calculated for the whole series of 21 samples.

## RESULTS AND DISCUSSION

The results of the analyses for the constituents significantly correlated with quality are shown in Table 2. In considering the data in Table 2, one must bear in mind that the study is not primarily concerned with the absolute quantities of the several constituents of each grade. These values may vary from season to season depending upon several factors, such as variety, soil type, irrigation, fertilization, and cultural practices. The main concern is in comparing the grades.

TABLE 2.—PARTIAL COMPOSITION OF SEVERAL GRADES OF ONE CROP OF HICKS VARIETY FLUE-CURED TOBACCO

Grade	Ca	Mg	Hygroscopicity	Total N	Protein N	Total alkaloids	Nicotine	Total sugars (as glucose)	Reducing sugars (as glucose)	Ethanol extract
	%	%	%	%	%	%	%	%	%	%
X2L	4.65	0.64	10.69	1.79	0.79	1.80	1.74	15.50	14.95	34.01
X4L	5.95	0.72	9.71	1.76	0.75	2.04	1.82	8.37	7.76	25.32
X50	6.87	0.87	9.32	2.19	0.85	2.31	1.85	2.90	2.60	19.95
XND	7.20	1.04	9.31	2.24	0.84	2.12	2.07	1.40	1.17	15.54
C1L	3.76	0.60	13.22	1.42	0.67	1.29	1.18	21.60	18.70	45.30
C3L	4.47	0.68	11.51	1.50	0.66	1.64	1.58	19.80	14.02	41.22
C40	4.83	0.66	10.49	1.71	0.72	1.76	1.72	15.13	11.58	29.22
C3LG	4.66	0.92	10.86	1.92	0.84	2.11	2.06	14.52	9.34	29.74
C50M	5.14	0.97	10.56	1.82	0.77	1.91	1.85	12.38	8.71	26.26
B1L	2.82	0.65	12.60	1.62	0.68	1.57	1.51	21.79	17.46	44.99
B2L	2.87	0.68	10.32	1.46	0.70	1.58	1.53	21.54	16.05	43.96
B3L	2.59	0.69	9.96	1.36	0.62	1.48	1.41	21.94	19.33	45.58
B40M	3.47	0.69	9.86	1.71	0.77	2.14	2.06	20.99	15.10	45.11
B3LG	3.39	0.73	9.00	1.89	0.65	2.31	2.27	18.99	13.03	40.79
B4G	3.63	0.72	9.56	2.15	0.89	2.60	2.27	16.66	9.59	38.04
B6G	4.21	0.79	9.02	2.31	1.11	2.99	2.88	14.92	11.73	35.25
BT2L	3.00	0.86	10.19	1.63	0.67	1.84	1.75	21.34	19.58	47.63
BT30	2.85	0.75	10.54	1.74	0.74	1.97	1.91	20.89	18.08	39.74
BT40	3.45	0.81	9.31	1.70	0.76	2.22	2.13	19.78	17.14	38.55
T30	3.48	0.81	10.21	1.84	0.75	2.22	2.10	18.84	16.52	38.54
T6D	3.42	0.78	10.35	1.84	0.76	2.24	2.14	20.31	17.69	33.72
r =	-0.500*	-0.668**	+0.641**	-0.829**	-0.658**	-0.755**	-0.758**	+0.608**	+0.629**	+0.671**

\*Analytical data were calculated on a moisture-free and sand-free basis

r=Coefficient of correlation

\*\*Significant at 5% level

\*\*\*Significant at 1% level



Correlation coefficients, which were used to assess the relationship between the constituents and quality, indicate that the constituents can be grouped into the three classes proposed by other investigators (5,16): (a) the constituents which gave a positive correlation between content and quality; (b) the constituents which gave a negative correlation between content and quality; (c) the constituents which gave no significant correlation between content and quality.

#### Constituents and Properties Positively Correlated with Quality

##### *Hygroscopicity*

The best grades of the lug, cutter, and leaf groups had pronounced hygroscopic properties in that the two best grades of each respective group had the highest moisture-holding capacity. These pronounced differences in moisture-holding capacity were not apparent in the grades from the upper part of the plant.

##### *Carbohydrates and Ethanol Extract*

Generally, the percentages of total sugars, reducing sugars, and ethanol extract were highest in the L coloured grades and lowest in the G grades. The percentages of total sugars ranged from 1.40 for XND to 21.94 for B3L. The largest difference in the percentages of total sugars or reducing sugars within a group was found between X2L and XND of the lugs. Except for the C1L and C3L grades, sugars were generally higher in the leaves of the middle and upper part of the plant than in the leaves from the lower portion of the plant.

The present finding—that a relatively high content of sugars is one of the most important criteria for high quality flue-cured tobacco—is in agreement with other reports (1, 3, 5, 7, 14, 16, 19). However, it has been stated by Harlan and Moseley (11) that very high levels of sugar may result in an impairment of the taste quality.

As the ethanol extract contains sugars as well as acids and resins, the ethanol extract values were generally high in the grades having high percentages of total sugars and reducing sugars. Large differences in the percentages of ethanol extract occurred within groups: from 34.01 to 15.54 in the lugs; 45.30 to 26.26 in the cutters; 45.58 to 35.25 in the leaf; and 47.63 to 33.72 in the leaves from the upper part of the plant. The ethanol extract has a higher coefficient of correlation with quality than either total sugars or reducing sugars.

#### Constituents Negatively Correlated with Quality

##### *Mineral Constituents*

The calcium and magnesium contents were inversely correlated with quality, as the best grades generally contained a lower quantity of calcium and magnesium than the low quality grades. The percentage of calcium was high in the lower leaves and decreased to a low value in the best leaf grades.

The calcium and magnesium results are in accord with those of Askew *et al.* (1). They found that the calcium and magnesium contents were lowest in the leaves near the middle of the plant where they obtained the highest quality tobacco.

### *Nitrogenous Constituents*

Except for protein nitrogen, the (L) lightest coloured grades had a lower content of nitrogenous constituents than either the (O) medium or (G) green coloured grades. The percentages of total nitrogen ranged from 1.36 for B3L to 2.31 for B6G. The total nitrogen did not appear to be correlated with stalk position as low and high values were found in each group, depending on the colour of the grade. Except for the B3LG grade, the protein nitrogen values tended to follow the percentages of total nitrogen. The percentages of total alkaloids ranged from 1.29 for CIL to 2.99 for B6G. The content of total alkaloids attained a maximum in the G coloured leaf grades. Only small amounts of the primary and secondary amine alkaloids have been found in the Hicks variety.

Darkis *et al.* (8) considered the flavour and the taste of the smoke to be directly correlated with the content of nitrogenous constituents, and that flue-cured tobacco containing 1.70 to 2.30 per cent total nitrogen gave the most satisfying smoke. According to Mason and Lee (14) a desirable range for total nitrogen is from 1.5 to 2.0 per cent. Data in Table 2 show several grades having a lower content of total nitrogen than the lower limits of the desirable ranges (8, 14). The L coloured grades had a lower content of nicotine and total nitrogen than either the O or the G coloured grades. These results show the direct relationship between nicotine and total nitrogen obtained by Woltz *et al.* (20).

### **Constituents and Properties Not Correlated with Quality**

Coefficients of correlation were not significant between quality and the petroleum ether extract, sucrose, starch, ash, silica, potassium, phosphorus, chlorine, sulphur, burn, or pH. However, these constituents and properties, except for sulphur, were related to stalk position.

The content of sand-free ash, which was affected by stalk position, ranged from 20 per cent in the lower leaves to 9 per cent in the leaves from the middle and upper part of the plant. The silica content varied from approximately 1 per cent in the bottom leaves to approximately 0.2 per cent in the middle and upper leaves of the plant. The potassium content varied considerably among grades but the lug, cutter, and leaf groups all contained about 1 per cent. The potassium content decreased to about 0.7 per cent in the tip group, indicating potash adequacy for the plant (10). The content of phosphorus increased with ascending stalk position and reached a maximum of 0.12 per cent in the leaf group. The phosphorus content decreased to 0.08 per cent in the tip group which was also the level found in the cutter group.

A sulphur content of approximately 0.75 per cent was distributed uniformly throughout the plant. The pH values decreased from 5.45 in the lug and cutter groups through 5.3 in the leaf group to 5.1 in the tip group. These results agree with the findings obtained by Darkis *et al.* (5) that the most acid tobaccos, as measured by pH, were produced near the top of the stalk. The chlorine content, which was about 2 per cent in the lug group, decreased sharply to a minimum of 0.3 per cent in the leaf group and then increased to 0.7 per cent in the tip group. The duration of burn, which had been fairly constant in cigarettes made from the lug, cutter, and leaf groups, increased about 20 per cent in cigarettes from the tip group.

The percentage of sucrose, which was 0.5 per cent in the lug group, increased to 4.5 per cent in the cutter and leaf groups and then decreased to 2.5 per cent in the tip group. The starch content was approximately 3.4 per cent in the lower leaves and 4.8 per cent in the middle and upper leaves.

The petroleum ether extract of leaves, which decreased with ascending stalk position, ranged from approximately 7 per cent in the lug group to approximately 4 per cent in the tip group.

#### Ratios of Constituents Correlated with Quality

A number of investigators have used ratios to correlate chemical composition with quality of tobacco (16). These ratios are obtained by dividing the percentages of constituents directly related to quality by the percentages of constituents inversely related to quality. Accordingly, a high ratio

TABLE 3.—NUMERICAL VALUES OF FOUR RATIOS OF VARIOUS CONSTITUENTS OF SEVERAL GRADES OF ONE CROP OF HICKS VARIETY FLUE-CURED TOBACCO

Grade	Ratio (1)	Ratio (2)	Ratio (3)	Ratio (4)
X2L	8.4	8.7	3.0	10.0
X4L	4.4	4.8	1.7	5.4
X50	1.2	1.3	0.5	1.4
XND	0.5	0.6	0.2	0.6
CIL	13.2	15.2	4.5	15.3
C3L	9.4	13.2	3.4	11.4
C40	6.8	8.9	2.6	8.2
C3LG	4.9	7.6	1.8	6.0
C50M	4.8	6.8	1.8	5.8
BIL	10.8	13.5	4.1	12.8
B2L	11.0	14.8	3.7	13.4
B3L	14.2	16.1	5.0	17.3
B40M	8.8	11.9	3.1	11.2
B3LG	6.9	10.1	3.2	8.7
B4G	4.5	7.8	1.7	5.6
B6G	5.1	6.5	1.7	6.5
BT2L	12.0	13.1	4.7	14.7
BT30	10.4	12.0	3.9	12.8
BT40	10.1	11.6	3.6	12.9
T30	9.0	10.2	3.5	11.2
T6D	9.6	11.0	3.7	12.0
r =	+0.723**	+0.727**	+0.703**	+0.699**

r Coefficient of correlation

\*\*Significant at the 1% level

(1) % Reducing sugars (expressed as glucose)

% Total nitrogen

(2) % Total sugars (expressed as glucose)

% Total nitrogen

(3) % Reducing sugars (expressed as glucose)

% Proteins †

(4) % Reducing sugars (expressed as glucose)

% Total nitrogen - % Nicotine nitrogen

†Percentages of protein nitrogen multiplied by 6.25

indicates a good quality of tobacco. A large number of ratios of chemical components were statistically analysed in relation to quality. Four of these ratios are presented in Table 3.

The data (Table 3) show that ratios were higher for L coloured grades than either the O or the G coloured grades.

#### REFERENCES

1. Askew, H. O., R. T. J. Blick, and Joyce Watson. Flue-cured tobacco. IV. Effect of position on the plant on chemical composition of tobacco leaf. *New Zealand J. Sci. Technol.* 29:158-163. 1947.
2. Assoc. Off. Agr. Chemists. Official methods of analysis. 7th ed. Washington, D.C. 1950.
3. Blick, R. T. J. Physical and chemical characteristics of typical American and Nelson tobacco-leaf samples. *New Zealand J. Sci. Technol.* 25B:53-62. 1943.
4. Cundiff, R. H., and R. C. Markunas. Determination of nicotine, nornicotine, and total alkaloids in tobacco. *Anal. Chem.* 27:1650-1653. 1955.
5. Darkis, F. R., L. F. Dixon, F. A. Wolf, and P. M. Gross. Correlation between composition and stalk position of tobaccos produced under varying weather conditions. *Ind. Eng. Chem.* 28:1214-1223. 1936.
6. Darkis, F. R., E. J. Hackney, and P. M. Gross. Turkish tobaccos: Characteristics and chemical composition of imported types. *Ind. Eng. Chem.* 39:1631-1642. 1947.
7. Darkis, F. R., and E. J. Hackney. Cigarette tobaccos: Chemical changes that occur during processing. *Ind. Eng. Chem.* 44:284-291. 1952.
8. Darkis, F. R., L. A. Baisden, P. M. Gross, and F. A. Wolf. Flue-cured tobacco chemical composition of rib and blade tissue. *Ind. Eng. Chem.* 44:297-301. 1952.
9. Elliot, J. M., and L. S. Vickery. Ontario flue-cured tobacco soils and their fertilizer requirements. *Can. Dept. Agr. Pub.* 987. Ottawa, Ont. 1956.
10. Gribbins, M. F., J. J. Reid, and D. E. Haley. The distribution of potassium in bright leaf cigarette tobacco and its influence on the quality of the leaf. *J. Agr. Research* 63:31-39. 1941.
11. Harlan, W. R., and J. M. Moseley. *Encyclopedia of chemical technology*, pp. 242-261. Interscience Encyclopedia, Inc., New York, N.Y. 1955.
12. Harrell, T. G. Titrimetric determination of chloride in tobacco products. Paper presented before Tobacco Chemists Research Conference, Medical College Virginia, Richmond, Virginia. 1954.
13. Heinze, P. H., and A. E. Murneek. Comparative accuracy and efficiency in determination of carbohydrates in plant material. *Univ. Missouri Coll. Agr., Agr. Expt. Sta., Research Bull.* 314. 1940.
14. Mason, J. Y., and M. T. Lea. Evaluation of tobacco. Paper presented before Tobacco Chemists Research Conference, North Carolina State College, Raleigh, N. Carolina. 1955.
15. Perrin, C. H. Rapid modified procedure for determination of kjeldahl nitrogen. *Anal. Chem.* 25:968-971. 1953.
16. Phillips, M., and A. M. Bacot. The chemical composition of certain grades of type 11 American flue-cured tobacco. Relationship of composition to grade characteristics. *J. Assoc. Off. Agr. Chemists* 36:504-524. 1953.
17. Snedecor, G. W. *Statistical methods*. 4th ed. Iowa State College Press, Ames, Iowa. 1946.
18. Vickery, H. B., and A. N. Meiss. Chemical investigations of the tobacco plant. IX. The effect of curing and of fermentation of the composition of the leaves. *Conn. Agr. Sta. Bull.* 569. 1953.
19. Ward, G. M. Physiological studies with the tobacco plant. *Can. Dept. Agr. Tech. Bull.* 37. Ottawa, Ont. 1942.
20. Woltz, W. G., W. A. Reid, and W. E. Colwell. Sugar and nicotine in cured bright tobacco as related to mineral element composition. *Soil Sci. Soc. Amer. Proc.* 13:385-387. 1948.

# INFLUENCE OF WEATHERING ON DDT COVERAGE IN A POTATO SPRAY PROGRAM<sup>1</sup>

D. D. POND<sup>2</sup> AND D. CHISHOLM<sup>3</sup>

*Science Service, Canada Department of Agriculture*

[Received for publication May 17, 1957]

## ABSTRACT

Weathering caused most of the early reduction in deposits of DDT sprayed on potato foliage in experiments at Fredericton, N.B., in 1953 and 1954. During July and August plants in the field lost 26 to 63 per cent of initial DDT deposits within 24 hours after spraying. This was significantly greater than that lost by comparable plants under greenhouse conditions.

## INTRODUCTION

Stone (5) suggested that, in any spray program, attention should be given to the increase in leaf area resulting from rapid foliage growth. Kelley *et al.* (2) contended that, to be effective, sprays must be applied at intervals based not only on the presence and susceptibility of insects but also on the rate of increase of leaf surfaces, the most critical time being the period of rapidly expanding growth of young plants.

Since a knowledge of the persistence of insecticide deposits is of considerable importance in the proper spacing of spray applications, foliage coverage and persistence of residue in a normal potato spray program were studied at Fredericton, New Brunswick, in 1953 and 1954. The results of the study are reported in this paper.

## MATERIALS AND METHODS

The experimental area in which commercial cultural practices were followed consisted of a 2-acre field planted with Katahdin Foundation Seed. Sprays containing 5 lb. of 20 per cent DDT emulsion\* and 4 lb. of Basic-Cop\*\* per 100 gallons of water were applied uniformly to the plants with a power sprayer. Pressure was maintained at 400 lb. per square inch and the 8-row boom carried three nozzles per row. The plants were sprayed on July 8, 17, and 27 and August 6, 18, and 27 in 1953, and on July 16 and 26 and August 9 and 18 in 1954.

In 1953, samples of the top, middle, and bottom leaves were used in the estimation of DDT residues, each sample consisting of 100-leaf disks, 21 mm. in diameter. Samples were taken approximately 2 hours after spraying or as soon as the foliage was dry, 5 days after spraying, and 9 days after spraying or just before application of the next spray.

At field planting time in 1954, a seed piece was also planted in each of 420 pots. A group of five pots was placed at each of the intersections of the 42 rows and the two diagonals of the field. Immediately after each spray a pot from each intersection of row and diagonal, 84 in all, was removed to the greenhouse. These pots were set in vermiculite and watered

\*Shell Chemical Co. of Canada, Toronto 1, Ont.

\*\*Green Cross Insecticides, Montreal, Que.

<sup>1</sup>Contribution No. 3588, Entomology Division, and No. 356, Chemistry Division, Science Service, Department of Agriculture, Ottawa, Ont.

<sup>2</sup>Associate Entomologist, Field Crop Insect Section, Entomology Laboratory, Fredericton, N.B.

<sup>3</sup>Assistant Chemist, Chemistry Section, Science Service Laboratory, Kentville, N.S.

TABLE 1.—DDT DEPOSITS ON POTATO FOLIAGE, FIELD PLOTS, 1953. (AVERAGES OF TOP, MIDDLE, AND BOTTOM LEAVES —  $\mu\text{g./cm.}^2$ )

Date of spraying	Days after spraying			Mean <sup>2</sup>
	0 <sup>1</sup>	5	9	
July 17	0.5	0.1	0.1	0.23
27	0.8	0.4	0.2	0.47
August 6	2.5	0.6	0.6	1.23
18	2.4	1.2	1.2	1.60
Mean <sup>3</sup>	1.55	0.58	0.53	

<sup>1</sup>Two hours after spraying.<sup>2</sup>Least significant difference at  $P.05 = 0.84$ <sup>3</sup>Least significant difference at  $P.05 = 0.73$ TABLE 2.—DDT DEPOSITS ON POTATO FOLIAGE, FIELD PLOTS, 1954. (AVERAGES OF TOP, MIDDLE, AND BOTTOM LEAVES —  $\mu\text{g./cm.}^2$ )

Date of spraying	Hours after spraying						2 hr. before next spray	Mean <sup>1</sup>
	2	24	48	72	96	120		
July 16	2.1	0.8	0.6	0.5	0.4	0.4	0.1	0.70
26	1.7	1.3	0.8	0.6	0.5	0.6	0.1	0.80
August 9	1.1	0.6	0.6	0.7	0.7	—	0.2	0.65
18	1.9	2.2	1.4	1.9	1.2	—	1.2	1.63
Mean <sup>2</sup>	1.70	1.23	0.85	0.93	0.70	0.50	0.40	

<sup>1</sup>Least significant difference at  $P.01 = 0.17$ <sup>2</sup>Least significant difference at  $P.01 = 0.23$ TABLE 3.—DDT DEPOSITS ON POTATO FOLIAGE, GREENHOUSE, 1954. (AVERAGES OF TOP, MIDDLE, AND BOTTOM LEAVES —  $\mu\text{g./cm.}^2$ )

Date of spraying	Hours after spraying						2 hr. before next spray	Mean <sup>1</sup>
	2	24	48	72	96	120		
July 16	—	1.8	1.6	0.8	1.0	0.8	—	1.20
26	—	1.0	1.5	1.0	0.8	0.6	0.5	0.90
August 9	—	1.4	1.3	1.3	1.2	—	—	1.30
18	2.2	2.5	2.3	1.3	1.6	1.7	—	1.93
Mean <sup>2</sup>	2.20	1.68	1.68	1.10	1.15	1.03	0.50	

<sup>1</sup>Least significant difference at  $P.01 = 0.22$ <sup>2</sup>Least significant difference at  $P.01 = 0.29$



daily, care being taken to not wet the foliage. Samples, as in 1953, were taken in the field approximately 2 hours after spraying. In addition, the top, middle, and bottom leaves of both the greenhouse and field plants were sampled at 24-hour intervals during the first 5 days and, as far as possible, again on the ninth day after each application. After each spray a new group of pots was taken to the greenhouse and those previously brought to the greenhouse were discarded. One hundred disks, each 21 mm. in diameter, were collected per field sample and 50 disks per greenhouse sample.

The colorimetric procedure of Schechter *et al.* (4) was followed and the surfaces of both sides of the disks were used in calculating the DDT residues.

### RESULTS AND DISCUSSION

Table 1 shows the average amounts of residual DDT on foliage in 1953 immediately after, and 5 and 9 days after four spraying dates. No DDT was found on the foliage 5 and 9 days after the July 8th spray and, because of the condition of the plants, no samples were taken after the spray application of August 27. There was no significant difference in the deposits on the top, middle, and bottom leaves. The data suggest that DDT was less persistent when sprayed in July than in August, and that there was no significant loss between the fifth and ninth days after spraying.

Tables 2 and 3 show that the mean residues were consistently lower in the field than in the greenhouse. In the field, the greatest losses occurred in the first 48 hours after spraying; in the greenhouse, in the first 72 hours.

Stone (5) reported that as soon as a new leaf is formed the growth of the one below it is checked and the leaf takes on accelerated growth; moreover, during the period of vegetative growth the wave of acceleration passes up the stem from one leaf to the next above it. In the present investigation no significant difference was found in the residues on the top, middle, and bottom leaves in either 1953 or 1954. This would indicate that factors other than foliage development were mainly responsible for the loss of DDT.

The loss pattern found in this investigation suggests that, in evaluating a DDT spray as an eradicant, analysis of foliage samples taken shortly after spraying should provide an adequate indication of spray coverage. However, sprays applied as protectants should be evaluated by analysis of foliage samples taken at least 2 days after sprays are applied.

### ACKNOWLEDGEMENTS

The authors wish to express appreciation to J. Friesen, for statistical treatment of the data, and to R. F. Bishop, for assistance in the preparation of the manuscript.

### REFERENCES

1. Fernow, K. H., and S. H. Kerr. Leafroll control by the use of insecticides. *Amer. Potato J.* 30: 187-196. 1953.
2. Kelley, R. A., J. B. Adams, and P. H. Baird. Potato foliage development. *Amer. Potato J.* 30:29-34. 1953.
3. Kerr, S. H. Insect virus vector relationships respective to the control of plant virus spread. Ph.D. thesis, Cornell University. 1953.
4. Schechter, M. S., G. B. Soloway, R. A. Hayes, and H. L. Haller. Colorimetric determination of DDT. *J. Ind. Eng. Chem., Anal. Ed.*, 17:704-709. 1945.
5. Stone, Winona E. Normal growth of potato leaves in greenhouse and field. *J. Agr. Research* 46:565-578. 1933.

# AN ELECTRONIC SEED COUNTER

C. H. GOULDEN<sup>1</sup> AND W. J. MASON<sup>2</sup>

*Canada Department of Agriculture, Ottawa, Ontario*

[Received for publication April 13, 1957]

## ABSTRACT

An electronic device for counting seeds in the size range from flax to barley has been developed. The device consists essentially of a vibration feed which feeds the seeds individually to a counter. The impact of the falling seeds on a piezo-electric crystal gives rise to electrical impulses which are amplified and counted. The accuracy of counting is good. The speed of counting varies from 200 to 300, per minute.

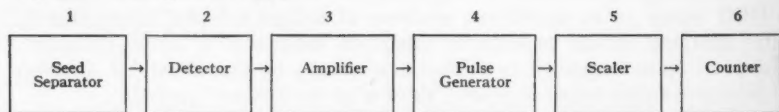
## INTRODUCTION

In plant breeding research, it is frequently necessary to count seeds. Counts are required chiefly (a) to determine the number of seeds per head, plant, or plot in breeding and genetic studies, and (b) to determine the average weight per seed of material being processed in the plant breeding nursery or of varieties or strains under test. Various other uses for seed counting occur, particularly in research on the quality of the final seed product.

A study of techniques for seed counting, with particular reference to cereal seeds, was initiated by the senior author in 1947 and continued up to the present time. Several devices tried out were reasonably successful but were discarded, mainly because they would be expensive to reproduce. The device described here combines reasonable cost with high counting speed, and accuracy.

## DESCRIPTION OF COUNTING DEVICE

The essential elements of the seed counting device are as indicated below; the arrows represent the direction of the passage of the electrical impulses through the system.



The seed-separating device is a modified Syntron\* vibrator Model EB-00, designed for the selective feeding of small objects for packaging. The power input requirement is 10 watts and the net weight 15½ lb. The device is shown in Figures 1 and 2. Alternating current at 115 volts is fed through a selenium rectifier to a variable 800 ohm, 50-watt resistor to provide control of the resulting pulsating d-c. voltage which operates the vibrator. The bowl of the original Syntron unit was modified to provide a narrower spiral inside ledge, along which the seeds move upward to the exit. This bowl is satisfactory for most cereal seeds, including flax. A slightly wider ledge may be required for large-seeded barley.

\* Syntron Limited, Montreal, Que.

<sup>1</sup> Director, Experimental Farms Service, Ottawa, Ont.

<sup>2</sup> Technician in charge of Instrument Shop, Central Experimental Farm, Ottawa, Ont.



FIGURE 1. Vibrator feed shown from the top to illustrate construction of ledge in feed bowl.

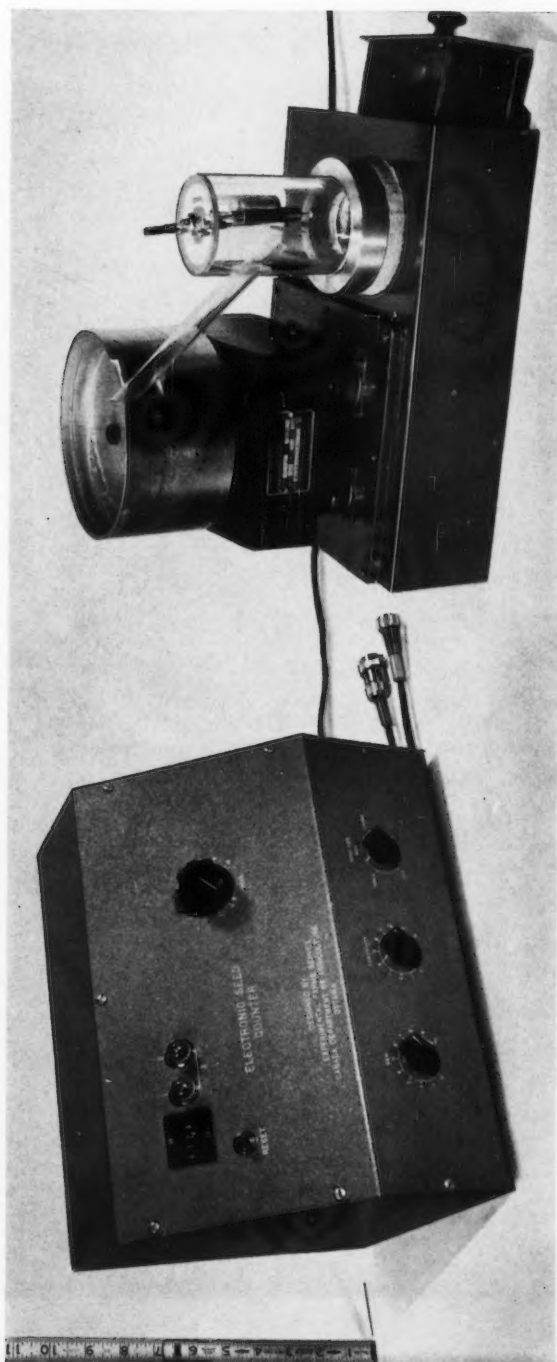


FIGURE 2. Vibrator feed and counting unit set up for normal operation.

From the separator the seeds fall through a plastic tube, as shown in Figure 2, and at the end of the fall strike a light metal plate about 1 inch in diameter which is attached to a shaft that substitutes for the needle in a phonograph crystal (Turner Model A). The electrical pulses that originate from the crystal as a result of the impact of a seed take the form of a series of waves typical of damped vibration. These are amplified and the leading edge operates a pulse generator which, in turn, operates the scaler and mechanical counter\*.

Figure 3 shows the schematic electrical circuit. The first tube (VI), a dual triode (12A×7), merely amplifies the impulses from the crystal. These are fed into the grid of a thyatron (2D21) in a circuit which acts as a pulse generator. It is triggered by the positive leading edge of the amplified impulses from the crystal, and the duration of the resulting positive pulse can be modified by means of the control R9. Changing R9 changes the RC time constant of the system ( $R9 + R8$ ) C5. It is designated on the front panel as the "Seed Size" control (Figure 2). The purpose of this control is to give adequate accuracy in counting seeds of various sizes. Large seeds give a longer train of pulses from the crystal than small ones. The control R9 must be set so that the output pulse from V2 lasts at least as long as the train of pulses arising from the impact of a single seed; otherwise, V2 would be activated a second time and a double count would result. Conversely, for small seeds, if R9 is set to give too long a pulse, one seed may follow another so rapidly as to arrive before the original pulse from V2 is completed, and would then fail to register.

In the pulse generator circuit, the negative bias on the grid can be varied from 0 to -25 volts by means of R26. The chief purpose of this is to allow for changing the circuit to a continuously operating pulse generator. This takes place at the point where the grid voltage is reduced below that required to prevent the 2D21 from firing. This is very convenient for checking the operation of the electrical circuit and the mechanical counter. The sensitivity control is turned to the left until the pulse generator starts to operate, as indicated by the neon lights on the scaler. The speed of pulse generation can be changed if necessary by adjusting the seed size control.

The reason for using the piezo-electric crystal as the basic device for obtaining electrical impulses from the falling seeds should be pointed out. Photo-electric counting was tried and found to work perfectly, except that nearly all seed samples contained light particles and small broken pieces which, with a photo-electric device, registered as seeds. With the method described, by judicious adjustment of R6, the amplifier control, and R26, the sensitivity control, it is possible to obtain a setting whereby most small pieces of seed and light pieces of other matter, such as straw or chaff, will not register a count.

The thyatron tubes V3, V4, V5, and V6 constitute a 1:4 scaler\*\*. It reduces the speed at which the mechanical counter (CTR) has to operate. The neon lights attached to the plates of V3 and V5 show on the front panel and indicate the exact digital count, according to the standard

\* Sodeco, Model TCeZ4E, 120v-5800~, Société des Comptures de Geneva. (Canadian agent: J. W. Ellis Industries, Toronto, Ont.)

\*\* Adapted from "Electronic Timer for Viscosity Measurements", U.S. National Bureau of Standards, Radio News,—Electronic Engineering Section, September, 1947.

binary system. Thus if, at the end of a count, 3 is registered on the neon lights and 44 on the counter, the total count is  $4 \times 44 + 3 = 179$ .

A more modern type of scaler can be substituted for the one mentioned here. However, the one described is not critical with respect to the values of the integral parts of the circuit and, since the thyratrons work at a minimum load, it should provide trouble-free operation and long life.

The last thyatron (V6) operates the mechanical counter as a portion of the plate circuit load.

The mounting of parts on a steel chassis is shown in Figure 4.

#### POWER SUPPLY

There are two special features of the power supply. In the first place, V8, a time delay switch with a delay time of 30 seconds, is inserted in the 150-volt tap from the bleeder R27. This is to prevent damage to the thyratrons due to the application of high voltage to the plates before the filaments are well heated. In the second place, an additional transformer winding is used in order to obtain bias voltages of 0 to -25 volts for V2 and -4.5 volts for the grids of the thyatron scaler tubes.

The high voltage winding of the power transformer is to provide 250 volts A.C. on each side of the centre tap. The winding for the bias voltage provides 25 volts a-c. and 10 milliamperes current.

#### DESIGN PROBLEMS

The chief problem in seed counting is to obtain efficient separation so that seeds can be fed individually to the counting mechanism. This presents difficulties because of variation in size and other characteristics within a given sample and the variable characteristics of different types of seed.

Wheat seeds are among the easiest to separate, but most samples vary to the extent that the largest seeds are three to four times the size and weight of the smallest seeds. In designing a groove for the vibration feed which will retain the larger seeds, it is obvious that it may still be possible for two small seeds to travel together and drop at the same time.

To maintain a maximum rate of feed, the ledge carries a surplus of seeds to the upper end where the ledge is narrowed and the extra seeds drop back into the bowl. At this point, the ledge is further modified into a groove to ensure that the seeds travel end to end (see Figure 1).

Oat seeds require a setting of the seed size control such that if one strikes the detecting plate on its end and flips over, giving a second impact before it leaves the plate, a double count will not be registered. This may require slowing down the counting speed.

Friction characteristics of seeds vary considerably both within and between types. Flax seeds are very slippery; other seeds are nearly spherical and roll rather than slide. In general, the surface of the groove in the feed bowl must provide appreciable friction with the surface of the seeds. Cast aluminum was the material used here and was found to be generally quite satisfactory.

Very small seeds, such as alsike clover, present a problem which has not yet been solved. It would seem necessary to use smooth material



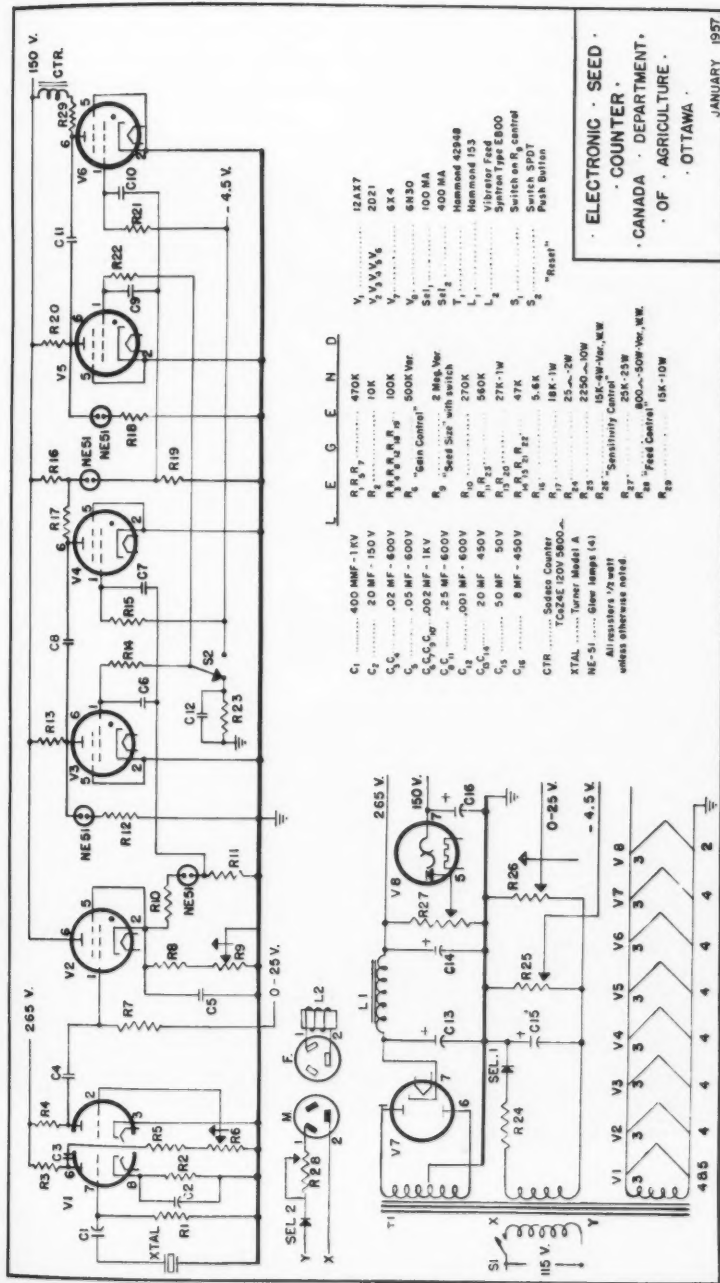


FIGURE 3. Schematic electrical circuit of seed counter.

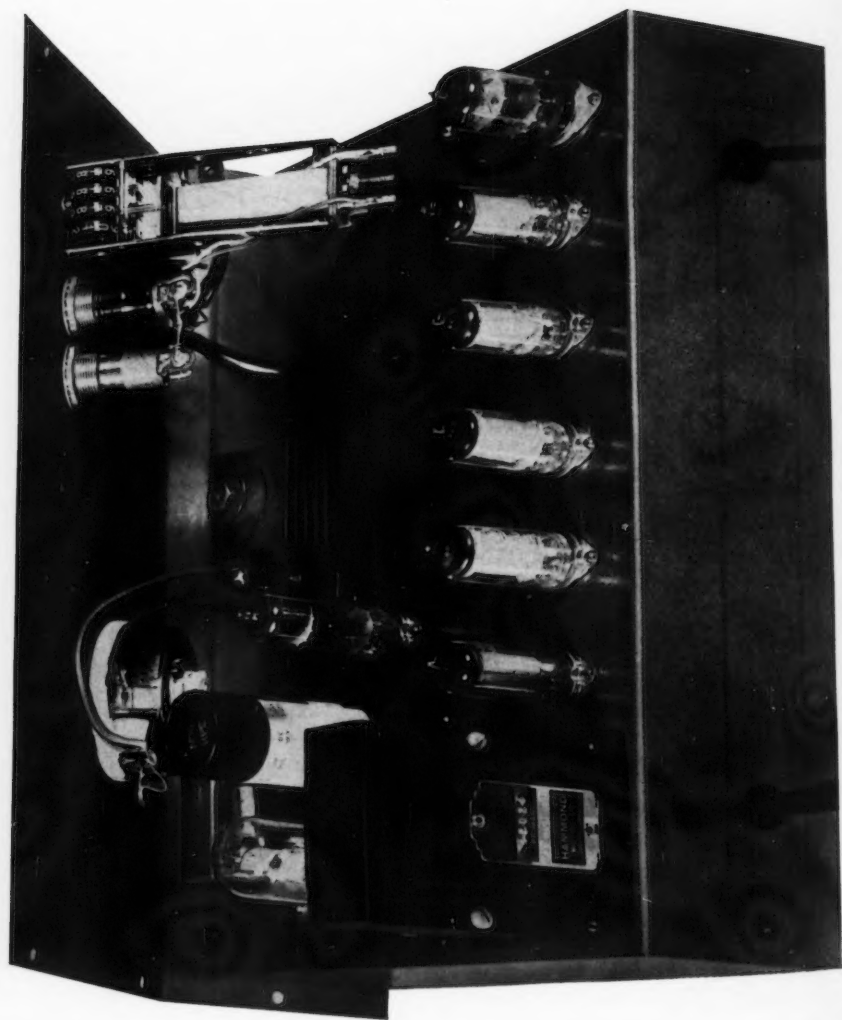


FIGURE 4. Illustrates mounting of parts of counter unit. Tubes in row from left to right are V<sub>6</sub>, V<sub>5</sub>, V<sub>4</sub>, V<sub>3</sub>, V<sub>2</sub>, 12A X7. *Upper left* shows vibration control and *upper right* mechanical counter and indication neon lights.

for the feed groove and to increase sensitivity. This is not merely a question of amplification but of both amplification and isolation of the detecting head from exterior vibration. It is obvious that if exterior vibration reaches the detecting plate and crystal spurious counts will result.

The device described operates successfully for seed in the size range from barley to flax. It is possible that, depending on physical characteristics, certain seeds smaller than flax may be counted.

### OPERATION

The counter may be set up as shown in Figure 2. It should be placed on a firm base and, normally, rubber pads or other vibration-reducing apparatus should not be used as they may lessen the efficiency of the vibrator feed. On starting the counter, a check is made on general operation by adjusting the sensitivity control, as pointed out above, to the point where the pulse generator operates continuously. It is then turned to the right, well past the point where oscillation ceases. The feed control is turned off while the bowl is being loaded, the neon lights brought to zero reading by means of the reset button switch, and the mechanical counter reset to zero. The feed control is then turned up to give a reasonable counting speed. The machine operates until the bowl is empty and the complete count is registered on the dials.

For making counts of 1,000 seeds, in order to obtain average seed weight, it is recommended that a given volume of seed, approximately 1,000 seeds, be taken and counted rather than manipulating the counter to give a count of exactly 1,000. For example, if 1,100 seeds are put in the bowl and the count stopped at 1,000, there is a possibility that the action of the vibrating bowl may have been such as to promote the feeding of the larger seeds; this will destroy the validity of the determination.

Counting speed will vary with the type of seed counted. Wheat seed can be counted at a speed of approximately 300 per minute and oats at about 200 per minute.

## MALTING QUALITY OF CANADIAN BARLEYS. VII. PARKLAND<sup>1</sup>

V. M. BENDELOW<sup>2</sup>, W. O. S. MEREDITH<sup>3</sup> AND W. H. JOHNSTON<sup>4</sup>

[Received for publication June 3, 1957]

### ABSTRACT

The variety Parkland, which was licensed for sale in Canada in 1956, is equal to O.A.C. 21 in malting quality. This variety was tested for 6 years under a wide environmental range in Western Canada and is similar to O.A.C. 21 in barley, malting and malt properties. Parkland has the advantage of yielding a higher malt extract.

Parkland is the first malting barley to be produced in Canada by the co-ordinated efforts of the plant breeder and cereal chemist at all stages of development. Prediction and malting tests proved valuable in providing the plant breeder with information on parents and hybrid lines, thus enabling him to make better selections in his crossing program. Details of quality testing of the various parents and the variety are given.

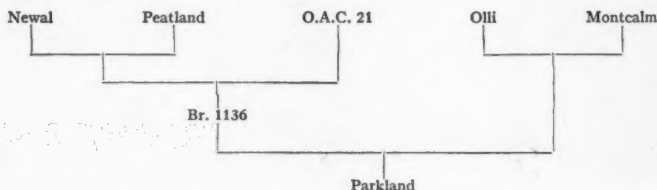
### INTRODUCTION

Parkland is a new barley variety produced at the Brandon Experimental Farm and its agronomic characters have been described by Johnston and Metcalfe (7). Parkland is equal to O.A.C. 21 in malting quality and is eligible for Grades 1 and 2 C.W. 6-row barley. As commercial shipments of Western Canadian barley in 1958 will probably contain carlots of Parkland, information on its malting quality is presented in this paper.

Quality tests were made at each stage in the development of Parkland, which is the first malting barley variety to be produced by a coordinated program of plant breeding and quality testing. Information on the progress of these tests is also presented. The data are given chronologically and confirm the utility of barley analyses as a method of predicting malting quality. They also serve to demonstrate the advantage of coordination between barley chemist and plant breeder in a breeding program for malting barley.

### MATERIALS

The parents of Parkland, in order of use in the breeding project, are: Newal, Peatland, O.A.C. 21 (the standard of malting quality for 6-row varieties), Olli and Montcalm. These varieties are shown in the following genealogical diagram:



<sup>1</sup>Contribution from the Cereal Crops Division, Canada Department of Agriculture, and the Grain Research Laboratory, Board of Grain Commissioners for Canada. Paper No. 218 of the Cereal Crops Division; No. 162 of the Grain Research Laboratory.

<sup>2</sup>Barley Chemist, Cereal Breeding Laboratory, Winnipeg, Man.

<sup>3</sup>Chemist, Grain Research Laboratory, Winnipeg, Man.

<sup>4</sup>Senior Agronomist (Cereals), Experimental Farm, Brandon, Man.

Laboratory malting tests and barley analyses (prediction tests) were made on all the parent varieties in the course of studies on the malting quality of Canadian barleys (3, 8). Malting tests were made on lines from the Newal  $\times$  Peatland cross, and malting and prediction tests were made on lines from the (Newal  $\times$  Peatland)  $\times$  O.A.C. 21 cross, the Olli  $\times$  Montcalm cross and the Br. 1136  $\times$  (Olli  $\times$  Montcalm) cross. The years in which the tests were made are shown in Tables 1 and 2. The data for the parental varieties were obtained over a period of years and from a number of stations, and the hybrid material was usually grown at Brandon and tested for one year.

Prediction tests on the final cross began in 1950 and the first laboratory malting test was made in the following year on samples grown at Brandon. Subsequently, malting tests of Parkland were made on material grown at several points in Western Canada.

### METHODS

#### Malting Tests

The laboratory malting test uses a 250-gram sample that is processed in experimental equipment (2, 4, 10) and the malts are analysed by standard procedures (1). Included in the information from the malting test are data on the yield of heavy grade barley and the 1000-kernel weight and nitrogen content of the sample (4). Malt analyses normally consist of malt extract yield, wort nitrogen content and saccharifying activity. In the final testing program on Parkland, additional determinations on barley and malt were made. These were the salt soluble and alcohol soluble nitrogen contents of the barley (11) and the cold water extract (9), viscosity of wort prepared by hot water extraction (9) and the alpha amylase activity (1) of the malt.

#### Prediction Tests

Extract yield and saccharifying activity are determined on barley samples. These analyses were developed in the course of extensive studies on malting quality (3, 5, and papers cited therein). The methods have been recently examined and again described by Bendelow and Meredith (6).

TABLE 1.—SUMMARY OF BARLEY QUALITY CHARACTERISTICS OF PARENTAL VARIETIES AND INTERMEDIATE HYBRIDS. DATA EXPRESSED AS DEVIATIONS FROM VALUES FOR O.A.C. 21

Variety	Year	1000 k.w.	Nitrogen	Extract	Sacch. activity
		gm.	%	%	
O.A.C. 21	1938	31.8	2.08	75.9	186
Newal	1938	2.4	0.09	-0.4	90
Peatland	1938	-2.6	0.26	-0.1	35
Olli	1938	-2.2	-0.08	1.3	30
Montcalm	1941, 1945	0.5	-0.16	1.8	4
Newal $\times$ Peatland (NXP) $\times$ O.A.C. 21			no data		
range of 20 lines	1942	-2.5 to 2.5	-.22 to .10	-1.6 to 2.0	-67 to 19
Br. 1136		1.9	-0.04	1.3	-14
Olli $\times$ Montcalm					
range of 19 lines	1943	0.1 to 6.5	0 to .46	2.7 to 1.9	-2 to 94
Olli $\times$ Montcalm $\times$ Br. 1136*	1950	-6.9 to 10.5	-.82 to .01	2.1 to 7.8	-134 to 22
range of 45 lines					
Br. 3833	1950	8.5	-0.42	7.1	-88

\*Check sample of O.A.C. 21 badly rusted and poor in quality.

TABLE 2.—SUMMARY OF BARLEY AND MALT CHARACTERISTICS OF PARENTAL VARIETIES AND INTERMEDIATE HYBRIDS.  
DATA EXPRESSED AS DEVIATIONS FROM VALUES FOR O.A.C. 21

Variety	Year	Plump barley	1000 kernel weight	Nitrogen	Malt extract	Sacch. activity	Wort nitrogen
		%	gm.	%	%		%
O.A.C. 21	1938-1940	74.5	32.2	2.23	74.4	131	1.14
Neval	1938-1940	17.2	3.3	0.10	-1.8	30	-0.06
Peatland	1938-1940	-11.7	-3.4	0.32	-1.1	6	0.08
Olli	1938-1940	-3.7	-1.1	-0.05	2.7	18	0.06
Montcalm	1939-1945	6.3	0.6	-0.13	1.0	7	-0.09
Neval X Peatland range of 14 lines	1938	-9.9 to 12.4	-3.2 to 1.2	0.18 to 0.52	-5.5 to -0.4	-23 to 40	-0.14 to 0
(N X P) X O.A.C. 21 range of 5 lines	1943	3.5 to 12.0	-1.4 to 1.4	-0.06 to 0	0 to 0.6	-20 to 0	-0.20 to -0.02
Br. 1136	1943	12.0	1.2	-0.02	0.4	-20	-0.18
Olli X Montcalm range of 6 lines	1944	-4.4 to 1.8	-1.8 to 1.8	-0.38 to -0.22	1-0.8 to 1.6	-19 to 26	-0.18 to -0.10
Olli X Montcalm X Br. 1136 range of 29 lines	1951	-5.3 to 12.5	-3.7 to 7.8	-0.19 to -0.11	-0.8 to 3.5	-54 to 64	-0.11 to 0.09
Br. 3833	1951	6.6	1.1	-0.16	1.8	6	0.01



### *Operational*

The results of prediction and malting tests were used by the plant breeder in the manner suggested by Anderson, Meredith and Sallans (3). The most promising lines selected by the plant breeder from the Newal × Peatland cross were malted in 1938 (the prediction test was being developed at that time) and selections made on the basis of quality were crossed with O.A.C. 21. Progeny from this cross were tested by the prediction method in 1942 and the best were malted in 1943. Results showed the most promising to be line Br. 1136. At this time some Olli × Montcalm selections were being examined for malting quality by both test methods and the best of this set were crossed with Br. 1136. Prediction tests were made in 1950, and malting tests in 1951, on the progeny of this cross. Line Br. 3833 proved to be the most promising and it was advanced into the Manitoba Regional Barley Test and the Western Co-operative Barley Test in 1953. Malting quality studies were continued each year until 1956 on material from these agronomic data. Additional malt analyses were made in the later stages of the program and samples grown in 1/10-acre plots were tested in a pilot brewery.

## RESULTS AND DISCUSSION

### *Parents and Intermediate Hybrid Lines*

A summary of the quality characteristics of the parent varieties and the intermediate hybrids is given in Tables 1 and 2. The first cross made in the series that eventually produced Parkland was Newal × Peatland, designed to combine straw strength and kernel size with the rust resistance of Peatland. Newal has somewhat larger kernels than the average, and is characterized by a very high barley saccharifying activity; but it does not modify well during malting. Peatland is characterized by smaller than average kernels, high nitrogen content and poor modification and produces a malt low in yield of extract. Quality tests, in 1938, on 14 lines from this cross indicated that there was little hope of recovering a malting barley (see Table 2). The barleys were generally high in nitrogen content and their modification during malting was inferior. Selections from this group were crossed with O.A.C. 21, the standard of malting quality, and the progeny were given prediction tests in 1942 (Table 1) and malting tests in the following year (Table 2). O.A.C. 21 has medium sized kernels with a medium nitrogen content in comparison with the average values for Canadian barley, and it modifies well during malting. Some of the (Newal × Peatland) × O.A.C. 21 lines had satisfactory kernel size, moderate nitrogen content and satisfactory extract, but the saccharifying activity tended to be too low (Tables 1 and 2). Therefore, a parent with high saccharifying activity was required in order to incorporate this property.

Olli has proved to be a good parent for providing enzymatic activity, but it usually produces small, thin kernels when grown outside of Alberta. It is, however, high in extract and saccharifying activity and modifies well during malting. The variety Montcalm closely resembles O.A.C. 21 in malting characteristics, but is higher in barley and malt extract. Progeny from an Olli × Montcalm cross were tested for quality characteristics in 1943 and 1944 (Tables 1 and 2) and some very promising selections were identified. One of these, chosen for its agronomic superiority, was crossed

with Br. 1136, the most promising line from the (Newal  $\times$  Peatland)  $\times$  O.A.C. 21 cross, in order to improve the low saccharifying activity. Quality tests were made on the resulting lines in 1950 and 1951 and Br. 3833 was selected as the best (Table 2).

This illustration typifies the method of testing hybrid selections used to this stage in the development of a malting variety. While prediction tests cannot be used to make final assessments of malting quality, they are useful for indicating the direction that the breeding program should take and their value to the plant breeder was stressed by Johnston and Metcalfe (7).

#### *Parkland*

Line Br. 3833, later named Parkland, was advanced to the Manitoba Regional Test and the Western Co-operative Barley Test in 1953. Over a period of 6 years, 32 pairs of samples of O.A.C. 21 and Parkland were given laboratory malting tests. The samples were grown at the Experimental Farm, Brandon, and in the two co-operative test projects (see Table 3). The results of the malting tests are summarized in Tables 3 and 4. Table 3 lists the barley and malt data for Parkland expressed as differences from O.A.C. 21 values and Table 4 shows the results of additional malt analysis studies.

The mean values at the foot of Table 3 indicate that Parkland and O.A.C. 21 are similar in malting characteristics, except that Parkland is higher than O.A.C. 21 in yield of heavy grade barley and in malt extract, and lower in barley nitrogen content. All of these are advantages in a malting barley. The comparisons between the two varieties are consistent from year to year. The variations from the mean differences in analytical values are lower than the errors of sampling and analysis (3). Thus the consistency in behaviour of Parkland in relation to O.A.C. 21 is obvious.

Measurements made during experimental malting showed that Parkland requires the same time as O.A.C. 21 to reach 44 per cent moisture content during steeping, which is an additional advantage in malting.

The close similarity between Parkland and O.A.C. 21 is further emphasized by the data in Table 4, which gives the results of additional barley and malt analyses. The two varieties are similar in distribution of barley protein, as measured by alcohol soluble and salt soluble nitrogen. They are

TABLE 3.—SUMMARY OF BARLEY AND MALT CHARACTERISTICS OF PARKLAND.  
DATA EXPRESSED AS DEVIATIONS FROM VALUES FOR O.A.C. 21

Test location	No. of stations	Year	Heavy grade	1000 k.w.	Barley N.	Wort N.	Malt extract	Sacch. act.
			%	gm.	%	%	%	
Brandon	1	1951	6.6	0.9	-0.16	0.01	1.8	6
Brandon	1	1952	1.1	-1.0	-0.22	-0.10	1.9	-19
Brandon	1	1954	12.9	0.1	-0.02	-0.06	1.2	-8
Manitoba Regional	3	1953	12.6	0.4	-0.16	-0.12	1.1	-20
Manitoba Regional	2	1954	20.5	1.5	-0.05	0	1.9	10
Manitoba Regional	2	1955	5.7	0.4	-0.11	0.08	0.7	15
Western Co-operative	6	1953	9.1	0.6	-0.09	-0.02	1.2	0
Western Co-operative	4	1954	17.5	1.5	-0.09	-0.01	1.8	-8
Western Co-operative	6	1955	4.5	0.6	-0.14	-0.02	1.8	-9
Western Co-operative	6	1956	7.1	1.0	-0.12	-0.05	2.1	2
Mean values, 32 samples								
Parkland			9.5	0.8	-0.11	-0.03	1.6	-3
O.A.C. 21			77.4	32.8	2.41	1.20	73.1	154

TABLE 4.—COMPARATIVE VALUES OF ADDITIONAL MALT PROPERTIES FOR O.A.C. 21 AND PARKLAND, 1954 AND 1955

Variety	Malt				Barley	
	Cold water extract	Cold wort nitrogen	Alpha amylase	Hot extract viscosity	Alcohol soluble nitrogen	Salt soluble nitrogen
	%	%	20° D.U.	c.p.	%	%
O.A.C. 21	19.2	0.67	32	1.67	Four stations	
Parkland	19.4	0.70	31	1.66		
O.A.C. 21	18.8	0.57	43	34.1	Six Stations	24.1
Parkland	19.6	0.62	43	32.4		23.9

also similar in the amount of cold water extract of malt, nitrogen content of this extract and in malt alpha amylase activity. All these factors are related to ease of modification of the barley constituents during malting, so that the two varieties are similar in growth pattern.

Results obtained from pilot brewing tests on Parkland and O.A.C. 21 showed that the two barleys had similar processing characteristics, with no indications of abnormalities, and produced closely similar beers. Thus Parkland was considered to compare favourably with O.A.C. 21 in brewing properties.

The results discussed above indicate that O.A.C. 21 and Parkland are closely similar in barley malting and malt properties, and any differences are in favour of Parkland. The two varieties were compared in an extensive series of tests over several years at a number of locations in Western Canada. Thus a wide range of environmental conditions were represented, as well as a reasonable range in barley nitrogen level. There is every reason to believe that Parkland will perform as well under widespread cultivation as it has done under test conditions, and the similarity in properties between the new variety and the accepted standard variety gives every assurance that industrial users will experience no difficulties in handling Parkland.

#### REFERENCES

1. American Society of Brewing Chemists. Methods of analysis. 4th rev. ed. 1944.
2. Anderson, J. A., and W. O. S. Meredith. Laboratory malting. III. Steeping equipment and method. *Cereal Chem.* 17:66-72. 1940.
3. Anderson, J. A., W. O. S. Meredith, and H. R. Sallans. Malting quality of Canadian barleys. IV. A summary of information of special interest to plant breeders. *Sci. Agr.* 23:297-314. 1943.
4. Anderson, J. A., and H. Rowland. Modified equipment and methods for the routine malting test and a study of its precision. *Sci. Agr.* 17:742-751. 1937.
5. Anderson, J. A., H. R. Sallans, and W. O. S. Meredith. Varietal differences in barleys and malts. XII. Summary of correlations between 18 major barley, malt and malting properties. *Can. J. Research, C*, 19:278-291. 1941.
6. Bendelow, V. M., and W. O. S. Meredith. Reliability of prediction tests for malting quality of barley. *Can. J. Agr. Sci.* 35:252-258. 1955.
7. Johnston, W. H., and D. R. Metcalfe. The history, description and performance of Parkland, a new malting barley. *Agr. Inst. Rev.* XI (5):11-14. 1956.
8. Meredith, W. O. S. Malting quality of Canadian barleys. V. Summary of seven years' tests on Montcalm, a new smooth-awned variety. *Sci. Agr.* 26:560-565. 1946.
9. Meredith, W. O. S., and V. M. Bendelow. Additional criteria of malting quality in varietal studies. *Proc. Amer. Soc. Brewing Chem.* 1956, pp. 77-82. 1956.
10. Meredith, W. O. S., K. Hlynka, and H. R. Sallans. Laboratory malting. IV. A germination chamber for routine malting tests. *Cereal Chem.* 21:261-258. 1944.
11. Rose, R. C., and J. A. Anderson. Fractionation study of barley and malt proteins. *Can. J. Research, C*, 14:109-116. 1936.

## INFLUENCES OF SOME FUNGICIDES ON ORCHARD MITES IN BRITISH COLUMBIA<sup>1</sup>

C. V. G. MORGAN<sup>2</sup>, N. H. ANDERSON<sup>3</sup> AND J. E. SWALES<sup>4</sup>

[Received for publication April 26, 1957]

### ABSTRACT

In apple orchards of the commercial fruit growing areas of British Columbia, nine fungicides gave the following results in the control of mites: a fungicide schedule consisting of four to seven sprays of lime-sulphur gave excellent control of *Metatetranychus ulmi* (Koch), but one spray applied at the pink stage gave control for only 2 months. Three summer sprays of elemental sulphur did not affect *M. ulmi*, but controlled *Tetranychus telarius* (L.) and *Vasates schlechtendali* (Nal.). A schedule of ferbam gave good control of *Eotetranychus carpini borealis* (Ewing). Four to seven sprays of captan favoured increases in numbers of *M. ulmi* and *E. carpini borealis*. *M. ulmi* also increased in numbers after five applications of ziram. A schedule of glyodin did not control any species; in some cases the numbers of *M. ulmi* and *E. carpini borealis* increased after applications of this fungicide. A schedule of maneb controlled *M. ulmi* and *V. schlechtendali*, delayed the peaks in populations of *T. telarius* and *E. carpini borealis*, but allowed large numbers of *Bryobia arborea* M. & A. Manam compared favourably with maneb against *M. ulmi* and *T. telarius*. One spray of Karathane controlled any species if the application was timed correctly.

Predatory mites, *Typhlodromus* spp., were not affected by glyodin but were reduced in numbers by ziram and nearly eliminated by maneb and Karathane. *Mediolata* sp. was slightly reduced in numbers by glyodin but maneb was somewhat more toxic and Karathane eliminated it. One spray of Karathane killed only 27 per cent of *Stethorus picipes* Csy.

Ferbam, maneb, and Karathane promoted tree vigour; large, healthy, dark-green leaves were characteristic of trees sprayed with these fungicides.

### INTRODUCTION

The British Columbia apple grower is confronted with the control of two fungus diseases: apple powdery mildew, *Podosphaera leucotricha* (Ell. & Everh.) Salm., and apple scab, *Venturia inaequalis* (Cke.) Wint. Two fungicides, lime-sulphur and wettable sulphur, commonly used to control these diseases, are known to reduce materially the numbers of the European red mite, *Metatetranychus ulmi* (Koch), the brown mite, *Bryobia arborea* M. & A., the apple rust mite, *Vasates schlechtendali* (Nal.), and the two-spotted spider mite, *Tetranychus telarius* (L.).

Control of mites with fungicides would offer a method of reducing the cost of the orchard spray program. Mites that over-wintered in the egg stage are most easily controlled with pre-bloom sprays, so that a fungicide having acaricidal properties and used during this period may provide good mite control. The material need not be a highly lethal acaricide, because a number of applications are usually necessary for fungus control.

In addition to their direct or indirect effects on phytophagous mites, some fungicides are detrimental to beneficial arthropods (3, 4, 21, 22, 24). In areas where fungicides constitute a major portion of the orchard spray program, the selection of a fungicide that is relatively innocuous to

<sup>1</sup> Contribution No. 3582, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Ont., in co-operation with the British Columbia Department of Agriculture, Victoria, B.C.

<sup>2</sup> Associate Entomologist, Entomology Laboratory, Science Service, Summerland, B.C.

<sup>3</sup> Assistant Entomologist; now at Entomology Laboratory, Science Service, Belleville, Ont.

<sup>4</sup> District Horticulturist, B.C. Department of Agriculture, Creston, B.C.

predatory species has materially alleviated the problem of controlling phytophagous mites (14). A knowledge of the effects of fungicides on beneficial arthropods is essential in the development of any spray program that attempts to harmonize the chemical and biological control of orchard pests.

The incidence of apple scab in the commercial fruit growing areas of British Columbia has increased in recent years and fungicides have been more widely used. The acaricidal properties of some of these materials, especially the organic fungicides, are not too well known. The objective of the investigations described in this paper was to evaluate these properties when fungicides were applied either in schedules for the control of apple scab or as single sprays for the control of apple powdery mildew.

#### MATERIALS AND METHODS

The major part of the work was done from 1951 to 1955 in the Kootenay Valley at Creston as a co-operative project of the Entomology and Plant Pathology Laboratories of Science Service, Canada Department of Agriculture, Summerland, and the B.C. Provincial District Horticultural Office at Creston. The test orchard consisted of three acres of McIntosh and Delicious apple trees. Each year the orchard was divided into six to eight non-replicated plots, which included a check plot not sprayed with a fungicide. When a fungicide was tested for more than one season it was applied to the same trees each year. The fungicides tested in this orchard included: lime-sulphur, ferbam, captan, glyodin, maneb, Manam, Karathane, Nirit, dichlone, zineb, and Orthorix. Depending upon weather conditions, four to seven fungicide applications were made each year as two to three pre-bloom sprays followed by two to four cover sprays. From 1951 to 1954, no insecticides or acaricides were applied to the plots until after the final application of the fungicides. In 1955, DDT was included with the first and second cover sprays. The sprays were applied with a hand-gun machine in 1951; in the following years a turbine-type concentrate machine was used.

Until 1952, the European red mite was the only serious mite pest in the Creston orchard; the two-spotted spider mite occurred sporadically. In August of that year the first record of the yellow spider mite, *Eotetranychus carpini borealis* (Ewing), in the Kootenay Valley was made in this orchard. Large numbers of the yellow spider mite developed in 1953, but very few were encountered in 1954 and 1955. Mite populations were estimated by sampling 10 leaves per tree from 5 or more trees per plot several times during the period of fungicide applications. In 1951 and 1952 the counts were obtained by the paper-impression or mangle method (27). In the following years the mites were removed from the leaves with a Henderson and McBurnie mite brushing machine and counted under a low-power microscope (7).

Experiments were conducted in 1953 in the Okanagan Valley at Penticton. The orchard consisted of an acre of mature McIntosh apple trees uniformly infested with the European red mite and the apple rust mite. Before the experiment was started the grower had applied a "pre-pink" spray of 50 per cent captan at 10 lb. per acre and 50 per cent DDT



at 8 lb. per acre. Thereafter each non-replicated plot was sprayed three times (May 13, calyx; May 29, first cover; June 12, second cover) with one of the following fungicides: sulphur, ferbam, captan, glyodin, and Iscothan. A non-sprayed check plot was maintained throughout the course of the experiment. The sprays were applied with a hand-gun machine. Mite counts were made several times during the season by the brushing machine method. At each date 10 leaves were sampled from each of 6 trees per plot.

Commencing in 1954, glyodin, maneb, and Karathane were tested in a block of Delicious apple trees at Summerland. Each non-replicated plot, consisting of 12 to 15 trees, was treated with one fungicide for two consecutive years, allowing study of the horticultural effects in addition to the influence on phytophagous and predacious mites. Four sprays were applied in 1954: "pink", calyx, and two cover sprays. In 1955, the same schedule was followed with the addition of a "pre-pink" spray. At each spray date the check plot was treated with water. All sprays were applied with concentrate machines. Populations of the brown mite, the European red mite, the two-spotted spider mite, the apple rust mite, *Typhlodromus* spp. [principally *T. occidentalis* Nesbitt and *T. fallacis* (Garman)], and *Mediolata* sp. were estimated four times in 1954 and five times in 1955. At each date, 20 leaves were sampled per tree from each of 8 trees in a plot and the mites removed from the leaves with a brushing machine. Ziram was tested in a similar way during 1955 in a nearby acre-block of Winesap apple, the trees not having been sprayed in 1954 and no other sprays having been applied in 1955.

Before 1951 some preliminary experiments were conducted at Oliver, Summerland, and Kelowna with lime-sulphur, nabam, and dinitro capryl phenyl crotonate (now sold as Karathane), applied either as "pink" or as summer sprays in non-replicated plots. The effectiveness of these materials for the control of the European red mite, the two-spotted spider mite, and the Mcdaniel spider mite, *Tetranychus mcdanieli* McG., was compared with that of parathion and a non-sprayed check plot. Mite populations were estimated several times during the season by sampling 10 leaves from each of 10 trees; counts were obtained by the paper-impression method.

The amounts of materials used in the above experiments are given in the tables.

TABLE 1.—AVERAGE NUMBERS\* OF THE EUROPEAN RED MITE PER LEAF ON THREE DATES AFTER LIME-SULPHUR AND PARATHION WERE APPLIED IN THE PINK STAGE OF DELICIOUS AND JONATHAN APPLE TREES, SUMMERLAND, 1948\*\*

Material	Amount per 100 gal.	June 2	June 21	July 26
Lime-sulphur <sup>1</sup>	1.25 gal.	0.0	0.4	8.4
Parathion, 25% <sup>2</sup>	4.8 oz.	0.0	0.0	2.6
Check	no spray	0.2	4.3	77.8

\*Based on the numbers of mites on 10 leaves per tree from each of 10 trees within one plot

\*\*Applied on May 11. 50% wettable DDT powder at 15 lb. per acre was also applied to all trees on June 4 and 24

<sup>1</sup> Liquid, 1.28 specific gravity (Oliver Chemical Co., Penticton, B.C.)

<sup>2</sup> Thiophos, wettable powder (American Cyanamid Co., New York, N.Y.)



## RESULTS AND DISCUSSION

*Lime-Sulphur*

Table 1 shows that a "pink" spray of lime-sulphur gave commercial control of the European red mite comparable to that given by parathion (before the development of parathion-resistant strains) applied at the same stage. The control lasted for over two months even in DDT-sprayed orchards, and under such conditions only a single summer acaricide would ordinarily be required to control this mite for the rest of the season.

Lime-sulphur applied in four or more applications for the control of apple scab gave excellent control of the European red mite and the yellow spider mite for the entire season (Table 2). Of the materials tested at Creston, lime-sulphur gave better control of these mites than the same number of sprays of glyodin, captan, or ferbam.

The acaricidal properties of lime-sulphur are well known. One or more correctly timed sprays control the blister mite, *Eriophyes pyri* (Pgst.) (26), other eriophyids (25), the European red mite (5, 18, 20), the two-spotted spider mite (20), and the brown mite (5, 20). It is generally considered to have little ovicidal action but it is fairly effective against the immature stages of most orchard mites (4-6, 20).

New organic fungicides are replacing lime-sulphur for the control of apple scab and apple powdery mildew in many areas, but in British Columbia lime-sulphur is still one of the recommended pre-bloom fungicides for these diseases. When applied in the pink stage of bud development it is an important acaricide for the control of the European red mite and the brown mite. At this period the winter eggs of the mites have practically all hatched and no summer eggs have been deposited. "Pink" sprays thus affect the mites when they are most vulnerable.

TABLE 2.—SEASONAL AVERAGE NUMBERS\* OF THE EUROPEAN RED MITE AND THE YELLOW SPIDER MITE PER LEAF ON MCINTOSH AND DELICIOUS APPLE TREES SPRAYED WITH VARIOUS FUNGICIDES, CRESTON, 1951-1953

Fungicide	Amount per application <sup>1</sup>			European red mite			Yellow spider mite 1953
	per 100 gal. 1951	per acre 1952	per acre 1953	1951	1952	1953	
Lime-sulphur <sup>2</sup>	2 gal.	8 gal.	8 gal.	0.7	2.1	4.2	0.2
Ferbam, 76% <sup>3</sup>	—	10 lb.	10 lb.	—	7.9	10.9	1.8
Captan, 50% <sup>4</sup>	—	10 lb.	10 lb.	—	13.5	4.3	21.9
Glyodin, 34% <sup>5</sup>	1 qt.	7.5 qt.	6 qt.	10.0	5.9	22.2	24.4
Check	no spray	no spray	no spray	10.8	7.6	34.5	9.9

\*Based on the numbers of mites on 10 leaves per tree on each of 5 to 10 trees within one plot sampled three to six times annually during June and July.

<sup>1</sup>Five applications ("pre-pink", "pink", calyx, 1st cover, 2nd cover) in 1951; four ("pink", calyx, 1st cover, 2nd cover) in 1952; seven ("pre-pink", "pink", calyx, and four covers) in 1953.

<sup>2</sup>Liquid, 1.28 specific gravity (Wynndel Co.-op., Wynndel, B.C.). Dinitroresol, 40% wettable powder at 3 lb. per acre, plus lime at 6 lb. per acre, included with the lime-sulphur in 1953.

<sup>3</sup>Fermate, wettable powder (Canadian Industries Ltd., Montreal, Que.).

<sup>4</sup>Orthocide, wettable powder (California Spray Chemical Corp., Richmond, Calif.).

<sup>5</sup>Crag Fruit Fungicide 341, liquid (Carbide and Carbon Chemical Corp., New York, N.Y.). Hydrated lime mixed with water in spray tank before addition of the glyodin, at 0.5 lb. per 100 gal. in 1951, 2.5 lb. per acre in 1952, and 1 lb. per acre in 1953.

### Elemental Sulphur

Summer applications of sulphur in British Columbia have given variable results but where it was used in three or more sprays in a fungicide schedule similar numbers of the European red mite occurred on sprayed and non-sprayed trees (Tables 2 and 3). Similar results were obtained where sulphur was used in conjunction with ferbam. At Creston, where these two materials were applied to the same trees for five consecutive years, European red mite populations were the same as or somewhat less than on the check trees. Though one or more DDT applications were made annually during this period, the two-spotted spider mite and the yellow spider mite were controlled by the sulphur.

The use of wettable or colloidal sulphur has been severely criticized by many workers because it promotes development of large populations of the European red mite; the effect is usually ascribed to the destruction of natural enemies (4, 21, 22). In Australia, Miller (16) considered sulphur of value in the control of the European red mite by preventing the population from increasing during the period of fungicidal applications. Summer sprays of sulphur are generally considered unsatisfactory for the control of the European red mite, but one or more applications control the two-spotted spider mite, the Pacific spider mite, *Tetranychus pacificus* McG., the yellow spider mite, the free-living eriophyids, and the brown mite (1, 5, 16, 17, 28).

### Ferbam

Four to seven applications of ferbam (iron dimethyldithiocarbamate) per year each at 10 lb. per acre apparently gave good control of the yellow spider mite at Creston (Table 2). Ferbam had no effect on the European red mite in 1952 but there was evidence of some control in 1953. At Penticton, three summer sprays had no effect on the European red mite or the apple rust mite (Table 3). However, the peak populations of the

TABLE 3.—SEASON AVERAGE NUMBERS\* OF PHYTOPHAGOUS MITES PER LEAF ON MCINTOSH APPLE TREES SPRAYED THREE TIMES (CALYX, 1ST COVER, AND 2ND COVER) WITH VARIOUS FUNGICIDES, PENTICTON, 1953

Fungicide	Amount per 100 gal. per application	European red mite	Apple rust mite
Sulphur, 69% <sup>1</sup>	4 lb.	12.0	8.1
Sulphur, 40% <sup>2</sup>	6 lb.	14.2	5.0
Ferbam, 76% <sup>3</sup>	2 lb.	16.3	15.4
Captan, 50% <sup>4</sup>	2 lb.	34.3	9.9
Glyodin, 34% <sup>5</sup>	1 qt.	25.8	23.0
Isocthan, 25% <sup>6</sup>	1 lb.	0.7	9.5
Check	no spray	15.0	17.2

\*Based on the numbers of mites on 10 leaves per tree on each of 6 trees within one plot sampled six times during May, June, and July

<sup>1</sup> Magnetic 70, paste: (Stauffer Chemical Co., Portland, Oregon)

<sup>2</sup> Colsul 40, paste (N.M. Bartlett Manufacturing Co. Ltd., Beamsville, Ont.)

<sup>3</sup> Fermate, wettable powder (Canadian Industries Ltd., Montreal, Que.)

<sup>4</sup> Orihocide, wettable powder (California Spray Chemical Corp., Richmond, Calif.)

<sup>5</sup> Crag Fruit Fungicide 341, liquid (Carbide and Carbon Chemical Corp., New York, N.Y.). Hydrated lime, 3 oz. per 100 gal., mixed with water in spray tank before addition of the glyodin

<sup>6</sup> Wettable powder (Rohm & Haas Co., Philadelphia, Pa.)

European red mite on the ferbam-sprayed plot occurred approximately two weeks later than on the check plot. In the tests, trees sprayed with ferbam were characterized by an unusually dark-green colour, large leaves, and increased terminal growth.

The effects of ferbam on phytophagous mites are not well known. In Nova Scotia, iron carbamates are very destructive to typhlodromid mites, and heavy European red mite infestations follow their use (14).

### *Captan*

The European red mite and the yellow spider mite increased in numbers on trees sprayed with captan (*N*-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide) except in one trial conducted at Creston in 1953 (Table 2). At Penticton, the European red mite increased to such an extent that two acaricidal sprays were required in the last two weeks of July to control the infestation (Table 3). A similar increase in the numbers of the yellow spider mite occurred after seven applications of captan in one trial conducted at Creston in 1953 (Table 2).

Captan, an excellent fungicide for the control of apple scab, is reported to have no detrimental effect on predacious mites (24). Conflicting results have been obtained on the effect of this material on phytophagous orchard mites. Kirby and McKinley (10) reported that it had a considerable ovicidal action on the summer eggs of the European red mite in laboratory trials in England. In contrast, Miller (16) found that significantly more mites occurred on captan-sprayed trees than on non-sprayed trees in Tasmania.

### *Glyodin*

Glyodin (2-heptadecyl-2-imidazoline acetate) showed no evidence of acaricidal action against the European red mite, the yellow spider mite, the two-spotted spider mite, or the apple rust mite (Tables 2-4). In some instances where glyodin was used in DDT-sprayed orchards, its action was evidently similar to that of captan. For example, in 1953 at Penticton, the numbers of the European red mite on glyodin-sprayed trees were similar to those on the check plot until after the first cover spray, but within six weeks the numbers had increased to an average of 50 per leaf in comparison with 25 on the check plot. At Creston in 1953, after seven applications of glyodin, the numbers of the yellow spider mite averaged 52 per leaf but only 11 per leaf occurred on the check plot. During this period the numbers of the European red mite on the two plots were approximately equal.

Where glyodin was the only material applied for two consecutive years at Summerland, the seasonal average numbers of all species of phytophagous mites were similar on the check and glyodin plots in both 1954 and 1955 (Table 4). However, in 1954 the peak population of the European red mite, which occurred in early August, was approximately twice as great on the glyodin plot as on the check plot. In this orchard it had no detrimental influence on *Typhlodromus* spp. but there were fewer of *Mediolata* sp.

TABLE 4.—SEASONAL AVERAGE NUMBERS\* OF PHYTOPHAGOUS AND PREDACIOUS MITES PER LEAF ON DELICIOUS AND WINESAP APPLE TREES SPRAYED WITH VARIOUS FUNGICIDES, SUMMERLAND, 1954 AND 1955

Fungicide	Amount per acre per application <sup>1</sup>	European red mite		Two-spotted spider mite		Brown mite		Apple rust mite		Typhlodromus spp.		Mediolata sp.	
		1954	1955	1954	1955	1954	1955	1954	1955	1954	1955	1954	1955
Glyodin, 34% <sup>2</sup>	5 qt.	16.2	4.4	6.6	0.9	9.7	8.1	438	337	0.02	0.40	0.00	0.63
Maneb, 70% <sup>3</sup>	10 lb.	1.8	2.2	6.9	1.6	18.2	24.1	68	38	0.00	0.03	0.05	0.14
Karathane, 25% <sup>4</sup>	5 lb.	2.8	2.7	12.2	1.4	0.3	0.2	289	42	0.00	0.03	0.00	0.00
Check, water	80 gal.	12.7	5.3	9.6	1.2	9.5	13.4	506	388	0.01	0.54	0.15	2.47
Ziram, 76% <sup>5</sup>	5 lb.	—	133.6	—	4.0	—	0.0	—	239	—	0.33	—	0.00
Check, water	75 gal.	—	62.6	—	1.6	—	0.0	—	262	—	1.72	—	0.03

\* Based on the numbers of mites on 20 leaves per tree on each of 8 trees within one plot sampled four times in 1954 and five times in 1955

<sup>1</sup> Four applications ("pink", calyx, 1st cover, 2nd cover) in 1954; five ("pre-pink", "pink", calyx, 1st cover, 2nd cover) in 1955

<sup>2</sup> Crag Fruit Fungicide 341, liquid (Carbide and Carbon Chemical Corp., New York, N.Y.). Hydrated lime, 10 oz. per acre, mixed with water in spray tank before addition of the glyodin in 1954; lime not used in 1955

<sup>3</sup> Dithane M-22, wettable powder (Rohm & Haas Co., Philadelphia, Pa.)

<sup>4</sup> Wettable powder (Rohm & Haas Co., Philadelphia, Pa.)

<sup>5</sup> Zerlate, wettable powder (E. I. du Pont de Nemours & Company Inc., Grasse Chemicals Dept., Wilmington, Delaware)

Glyodin, widely used in the eastern United States and in Canada for the control of apple scab, is particularly recommended in modified spray programs because it is innocuous to predacious arthropods and does not interfere with the natural control of the European red mite (3, 8, 18). Although the material is generally considered not to be acaricidal, Lienk and Chapman (13) obtained 96.6 per cent reduction in numbers of active stages of the European red mite three days after an application.

### Ziram

Five sprays of ziram (zinc dimethyldithiocarbamate) favoured large increases in populations of the European red mite. Seasonal numbers of eggs and active mites averaged 133.6 per leaf on ziram-sprayed trees as compared with 62.6 on the adjacent check plot (Table 4). Peak populations in early August of 436 per leaf caused considerable foliage injury. Although ziram was detrimental to the predacious mite *T. occidentalis*, the increase in numbers of the European red mite on the ziram-sprayed plot as compared with the check plot cannot be attributed entirely to the reduction of these predators. On June 10, after three applications of ziram, *T. occidentalis* averaged 3 per 100 leaves in comparison with 6 on the check plot. Ziram also favoured the development of the two-spotted spider mite, but it had no effect on the apple rust mite.

### Maneb

The experiments in 1953 to 1955 with commercial preparations of maneb (manganese ethylenebisdithiocarbamate) applied in a fungicidal spray schedule consistently gave good control of the European red mite and the apple rust mite (Tables 4 and 5). In the low infestations of the two-spotted spider mite and the yellow spider mite that occurred during these trials, maneb provided good control, or at least delayed the peaks in population by a month or more beyond that on non-sprayed trees. No visible foliage injury was caused by these two species of mites on the maneb-sprayed trees.

TABLE 5.—SEASONAL AVERAGE NUMBERS\* OF THE EUROPEAN RED MITE AND THE TWO-SPOTTED SPIDER MITE PER LEAF ON MCINTOSH AND DELICIOUS APPLE TREES SPRAYED WITH VARIOUS FUNGICIDES, CRESTON, 1954 AND 1955

Fungicide	Amount per acre per application <sup>1</sup>		European red mite		Two-spotted spider mite	
	1954	1955	1954	1955	1954	1955
Maneb, 70% <sup>2</sup>	10 lb.	10 lb.	3.4	3.4	0.7	0.1
Manam, 42% <sup>3</sup>	—	7 lb.	—	2.9	—	0.4
Karathane, 25% <sup>4</sup>	5 lb.	—	1.7	—	0.1	—
Check	no spray	no spray	58.0	42.7	2.0	3.2

\*Based on the numbers of mites on 10 leaves per tree on each of 10 trees within one plot sampled six times in 1954 and five times in 1955.

<sup>1</sup>Six applications ("pre-pink", "pink", calyx, and three covers) in 1954; seven ("pre-pink", "early pink", "late pink", calyx, and three covers) in 1955.

<sup>2</sup>Manzate, wettable powder (Canadian Industries Ltd., Montreal, Que.), in 1954; Dithane M-22, wettable powder (Rohm & Haas Co., Philadelphia, Pa.), in 1955.

<sup>3</sup>Wettable powder (Boots Pure Drug Co. Ltd., Nottingham, England)

<sup>4</sup>Wettable powder (Rohm & Haas Co., Philadelphia, Pa.)

One of the most striking features of maneb is its ineffectiveness against the brown mite. At Summerland, in 1954 and 1955, the brown mite population on the maneb plot was considerably higher than on non-sprayed plots or on plots sprayed with other fungicides (Table 4). The seasonal average numbers of brown mites were about equal to those on trees sprayed with DDT, but the rate of development followed a different pattern. On the DDT plot the numbers of the brown mite were highest during late July and early August and declined during the latter part of the season; on the maneb plot the population increased throughout the season and reached a peak in September.

Predacious mites occurred in the maneb plot, but the numbers were much lower than in the water-sprayed check plots. Maneb is apparently somewhat less toxic to *Mediolata* sp. than to *Typhlodromus* spp. as the former were present during most of the period of fungicide applications.

In these experiments maneb appeared to stimulate tree growth, resulting in large fruit and heavy crop production. Larger leaves, generally denser and darker foliage, and increased terminal growth were also evident. The unusually vigorous trees withstood the infestation of the brown mite with little evidence of foliage damage.

Maneb was shown to have acaricidal properties by Rich (23), but only when used with a wetting agent. R. S. Downing, Entomology Laboratory, Summerland, B.C., showed that a single spray of maneb, applied as an ovicide at the pre-pink stage or as a summer spray against active mites, gave no control of the European red mite.

#### *Manam*

At Creston, seven sprays of Manam (manganese dimethyldithiocarbamate) gave good control of the European red mite and the two-spotted spider mite, the only two mite species of importance in the orchard where the preparation was tested (Table 5). In this one experiment the acaricidal value of a fungicidal spray schedule of Manam compared favourably with that of maneb.

#### *Karathane*

Three to six annual applications of Karathane (dinitro capryl phenyl crotonate and other nitro phenol derivatives; formerly sold as Arathane, Iscothan, and Mildex) in a fungicide schedule gave good commercial control of the European red mite and the brown mite (Tables 3-5). Control of the apple rust mite varied from fair to good; in one instance good control was maintained until September, but thereafter the mites increased rapidly in numbers. Because this increase occurred so late in the season a summer mite spray was unnecessary. Karathane gave little control of the two-spotted spider mite in these experiments, probably because that species is a late-season pest, normally most numerous sometime after fungicidal spraying has been discontinued. As indicated in Table 6, a single summer spray applied specifically as an acaricide controlled the two-spotted spider mite and the Medaniel spider mite. A summer spray of Karathane also provides excellent control of the yellow spider mite.



TABLE 6.—AVERAGE NUMBERS OF TWO PHYTOPHAGOUS MITES AND ONE PREDACIOUS INSECT IMMEDIATELY BEFORE AND 12 DAYS AFTER KARATHANE AND PARATHION WERE APPLIED TO DELICIOUS, NEWTOWN, AND WINESAP APPLE TREES AT SUMMERLAND ON AUG. 31, 1950

Material	Amount per 100 gal.	Two-spotted and McDaniel spider mites <sup>1</sup>		<i>Stethorus picipes</i> <sup>2</sup>	
		Before spray	After spray	Before spray	After spray
Karathane, 25% <sup>3</sup>	1.5 lb.	46.4	3.4	93	68
Parathion, 15% <sup>4</sup>	1 lb.	22.3	0.3	110	3
Check	no spray	8.0	13.4	50	64

<sup>1</sup> Average numbers per leaf, from 10 leaves per tree on each of 10 trees within one plot

<sup>2</sup> Average numbers of larvae, pupae, and adults per 250 leaves, from 50 leaves per tree on each of 5 trees within one plot

<sup>3</sup> Wettable powder (Rohm & Haas Co., Philadelphia, Pa.)

<sup>4</sup> Thiophos, wettable powder (American Cyanamid Co., New York, N.Y.)

TABLE 7.—AVERAGE NUMBERS\* OF THE EUROPEAN RED MITE PER LEAF AFTER ARATHANE AND PARATHION WERE APPLIED IN THE PINK STAGE OF APPLE BUD DEVELOPMENT, OLIVER, SUMMERLAND, AND KELOWNA, 1949\*\*

Material	Amount per 100 gal.	Oliver June 24	Summerland June 27	Kelowna June 30
Arathane, 25% <sup>1</sup>	1.5 lb.	2.6	0.9	0.8
Parathion, 15% <sup>2</sup>	0.75 lb.	0.3	0.3	0.2
Check	no spray	46.9	13.9	25.7

\*Based on the numbers of mites on 10 leaves per tree on each of 10 trees within one plot

\*\*Applied on April 29 at Oliver, on May 2 and 3 at Summerland, and on May 5 at Kelowna. One to two cover sprays of DDT applied to all trees in each orchard before June 11

<sup>1</sup> Wettable powder (Rohm & Haas Co., Philadelphia, Pa.)

<sup>2</sup> Penphos W-15, wettable powder (Pennsylvania Salt Mfg. Co., Tacoma, Wash.)

Several tests proved that Karathane controls the European red mite when one spray of this fungicide is applied in the pink stage of apple bud development (Table 7). In every case, the control by Karathane compared favourably with that from parathion for approximately two months after the application.

Karathane was lethal to *Typhlodromus* spp. when applied in a fungicidal spray schedule, but it did not prevent their re-establishment late in the season (Table 4). It was very toxic to *Mediolata* sp., no specimens of which were collected in Karathane-sprayed trees during a 2-year period (Table 4). On the other hand, the coccinellid *Stethorus picipes* Csy. is rather tolerant of this chemical (Table 6). One summer application reduced the numbers of *S. picipes* by only 27 per cent whereas parathion killed 97 per cent. On non-sprayed trees the numbers of *S. picipes* increased 28 per cent during the same period.

Karathane, like maneb, increased tree vigour. In these experiments the size of Delicious apples from Karathane-sprayed trees averaged 26 per cent larger than fruit from non-sprayed trees. Other dinitro compounds have produced similar beneficial effects under British Columbia conditions (19).

The acaricidal properties of Karathane were first mentioned by Lathrop and Hilborn (12), who in 1950 showed that a dust containing 5 per cent of the active ingredient and applied in eight post-blossom sprays controlled the European red mite. Since then several workers have reported on its acaricidal properties (2, 9, 10, 15, 18).

#### *Other Fungicides*

No control of the European red mite resulted from three or more fungicidal applications of Nirit (thiocyano dinitrobenzene\*, dichlone (2,3-dichloro-1,4-naphthoquinone), or zineb (zinc ethylenebisdithiocarbamate). Four sprays of Orthorix (calcium polysulphides and polyethylene glycol monoisooctyl phenylether\*\*) were very effective. A summer spray of nabam (disodium ethylenebisdithiocarbamate) killed 56.3 per cent of the European red mite.

#### ACKNOWLEDGEMENTS

The authors wish to thank M. F. Welsh and D. L. McIntosh, Plant Pathology Laboratory, Summerland, for their co-operation and advice during this study. Thanks are due to G. Thorpe, Provincial District Horticultural Office, Creston, who did the spraying and sampling in 1951 and 1952, and to D. A. Chant, now of the Entomology Laboratory, Belleville, Ont., and Harry Dominique, Entomology Laboratory, Vancouver, B.C., for their laboratory assistance.

\*Kingsley & Keith Ltd., Montreal, Que.

\*\*California Spray Chemical Corp., Freewater, Oregon

#### REFERENCES

1. *Anonymous*. Recommendations for codling moth and orchard mite control in Washington for 1940. Wash. State Coll. Extension Bull. 252. 1940.
2. Chapman, P. J., and S. E. Lienk. Orchard mite control experiments in western New York. *J. Econ. Entomol.* 43:309-314. 1950.
3. Clancy, D. W. Effects of spray practices on apple pests and their natural enemies. *Mountaineer Grower* 25:11-21. 1955.
4. Collyer, E. Insect population balance and chemical control of pests. *Chem. & Ind.*, pp. 1044-1046. 1953.
5. De Onge, E. R. The preparation and use of colloidal sulphur as a control for red spider. *J. Econ. Entomol.* 17:533-538. 1924.
6. Eaton, J. K., and R. G. Davies. The toxicity of certain synthetic organic compounds to the fruit-tree red-spider mite. *Ann. Appl. Biol.* 37:471-489. 1950.
7. Henderson, C. F., and H. V. McBurnie. Sampling techniques for determining populations of the citrus red mite and its predators. *U.S. Dept. Agr. Circ.* 671. 1943.
8. Hilborn, M. T., and F. H. Lathrop. Organic fungicides in the control of apple scab and European red mite. *Phytopathology* 41:52-55. 1951.
9. Hofmaster, R. N., and D. E. Greenwood. Control of mites on strawberries in Virginia. *J. Econ. Entomol.* 46:224-233. 1953.
10. Kirby, A. H. M., and K. S. McKinlay. Laboratory experiments on the toxicity of potential acaricides. *Ann. Rept. East Malling Research Sta.*, 1950, pp. 164-171. 1951.
11. Lathrop, F. H. Apple insects in Maine. *Maine Agr. Expt. Sta. Bull.* 540. 1955.
12. Lathrop, F. H., and M. T. Hilborn. European red mite control. *J. Econ. Entomol.* 43:172-175. 1950.
13. Lienk, S. E., and P. J. Chapman. Evaluation of acaricides on orchard mites in 1952. *J. Econ. Entomol.* 46:1085-1086. 1953.
14. Lord, F. T. The influence of spray programs on the fauna of apple orchards in Nova Scotia. III. Mites and their predators. *Can. Entomologist* 81:202-214. 1949.

15. Madsen, H. F., and A. D. Borden. Pre-bloom treatments to control European red mite eggs on pears in northern California. *J. Econ. Entomol.* 48:103-105. 1955.
16. Miller, L. W. Effects of certain new fungicides on the European red mite in Tasmanian apple orchards. *Tasmania J. Agr.* 24:209-212. 1953.
17. Miller, R. L., W. W. Yothers, and I. P. Bassett. Iron sulphate and other materials for increasing the effectiveness of sulphur insecticides on citrus trees. *Proc. Florida State Hort. Soc.* 46:52-56. 1933.
18. Moore, M. H., and A. H. M. Kirby. Can the new organic fungicides help the fruit grower? *Ann. Rept. East Malling Research Sta., 1951*, pp. 200-204. 1952.
19. Morgan, C. V. G., and J. Marshall. Dinitrophenol derivatives as summer acaricides in British Columbia. *Sci. Agr.* 29:191-199. 1949.
20. Newcomer, E. J., and M. A. Yothers. Experiments for the control of the European red mite and other fruit-tree mites. *U.S. Dept. Agr. Tech. Bull.* 25. 1927.
21. Pickett, A. D. A critique on insect chemical control methods. *Can. Entomologist* 81:67-76. 1949.
22. Pickett, A. D., N. A. Paterson, H. T. Stultz, and F. T. Lord. The influence of spray programs on the fauna of apple orchards in Nova Scotia: I. An appraisal of the problem and a method of approach. *Sci. Agr.* 26:590-600. 1946.
23. Rich, S. Miticidal action of barium and manganese ethylene bisdithiocarbamates. *Phytopathology* 44:387. 1954.
24. Ripper, W. E. Effects of pesticides on balance of arthropod populations. *In Annual Review of Entomology*, Vol. 1, ed. by E. A. Steinhaus, pp. 403-438. Annual Reviews, Inc., Stanford, Calif. 1956.
25. Thompson, W. L. Lime-sulphur sprays for the combined control of purple scale and rust mites. *Univ. Florida Agr. Expt. Sta. Bull.* 282. 1935.
26. Treherne, R. C. Report from Vancouver district: insects economically important in the Lower Fraser Valley. *Proc. Entomol. Soc. Brit. Columbia* 4:19-33. 1914.
27. Venables, E. P., and A. A. Dennys. A new method of counting orchard mites. *J. Econ. Entomol.* 34:324. 1941.

# A NINETEEN-CHROMOSOME BARLEY PLANT<sup>1</sup>

A. MOCHIZUKI<sup>2</sup> AND E. REINBERGS<sup>3</sup>

*University of Manitoba, Winnipeg, Manitoba*

[Received for publication April 29, 1957]

## ABSTRACT

One plant with a somatic chromosome number of 19 was found in a population of 400 basically tetraploid plants of the barley variety O.A.C. 21. The morphology and cytology of this plant is described. Cytological observations indicate that the 19-chromosome plant was produced by the union of  $2n-2$  and  $n$  gametes.

## INTRODUCTION

One of the characteristics by which artificially-produced autotetraploids differ from the corresponding diploids is in the formation of multivalent associations during meiosis. In autotetraploid barley, quadrivalent associations are usually observed and the number of quadrivalents at first metaphase varies from one to four.

Abnormal disjunction of the quadrivalents will give unequal distribution of chromosomes to egg cells and pollen grains. Since some of the gametes with abnormal chromosome number survive in barley, a chance union of such gametes will produce aneuploids in the offspring. Chen *et al.* (1) found microspores with 12 to 16 chromosomes and pollen mother cells with 24 to 30 chromosomes. Aneuploid plants with chromosome numbers ranging from 26 to 31 have been reported by Dorsey (2), Müntzing (3) and Rosendahl (4). In addition to this range in number, a 24-chromosome plant was reported by Rosendahl (4). This plant produced seed. In general, however, the aneuploids were dwarfish, tillered profusely and did not produce heads.

The authors checked the chromosome numbers of the progenies of tetraploids derived from five barley varieties—Montcalm, Brant, O.A.C. 21, G.B. 61 at the  $C_4$  and Mochi-Mugi at the  $C_{14}$  generations—and also observed a large percentage of plants with abnormal chromosome numbers. Approximately 650 plants were studied and the chromosome numbers, with one exception, were observed to range from 26 to 31. One plant of O.A.C. 21 had only 19 chromosomes. Since this is a considerably lower number of chromosomes than hitherto reported, the morphology, cytology and the possible origin of this plant are described.

## ORIGIN OF 19-CHROMOSOME PLANT

The pedigree of the 19-chromosome plant can be traced back to colchicine-induced tetraploid O.A.C. 21 barley produced at the Ontario Agricultural College in 1954. The  $C_1$  progenitor did not differ from the other plants in that generation. It was 47 per cent fertile, producing 14

<sup>1</sup> Contribution from Division of Plant Science, University of Manitoba, Winnipeg, Man. (Rosner Research Chair in Agronomy, Publication No. 3).

<sup>2</sup> Post-doctorate Fellow, National Research Council of Canada.

<sup>3</sup> Graduate Student, University of Manitoba; now with Dept. of Field Husbandry, Ontario Agricultural College, Guelph, Ont.

seeds. In the  $C_2$  generation, 5 of these seeds were grown in pots in a greenhouse. Only two of the five plants set seed; the other three were dwarfs. One of the normal plants was 6.7 per cent fertile but did not produce viable offspring. The other normal plant was 20 per cent fertile and, from the seed produced, seven plants were obtained in the  $C_3$  generation. These seven plants were grown in the field in a 5-foot row adjacent to a diploid O.A.C. 21 barley row which was used as a check for fertility studies. The observed fertility of these seven plants ranged from 20 to 80 per cent.

The  $C_3$  plant, which was 20 per cent fertile, produced 6 seeds of which 5 germinated and were grown in separate pots in the greenhouse at the University of Manitoba. The 19-chromosome plant was identified in this population in October, 1956.

### MORPHOLOGICAL OBSERVATIONS

The size and shape of the 19-chromosome plant, compared with the normal tetraploid O.A.C. 21 barley, can be seen in Plate I, Figure 1.

The 19-chromosome plant was about half as tall as the normal tetraploids. The culms had four internodes which were short and the nodes never emerged from the leaf sheaths.

The plant produced three tillers, of which only one formed a spike in a normal manner. The other two tillers formed spikes at a much later date after the plant had been transplanted and the first tiller removed. Additional tillers were induced later by repeated transplanting. The first tiller was much later in maturity than the main tillers of normal tetraploids grown at the same time. It was still green four weeks after normal tetraploids matured. At this time it was removed in order to stimulate the development of the secondary tillers.

Seven leaves were produced on each one of the three tillers observed. The leaf width was approximately the same as that of normal tetraploids, but the length was only half that of normal tetraploids. The flag leaves of the later tillers were very short and broad. The colour of the leaves and the stem was dark green.

The spike did not emerge completely from the leaf sheath. In appearance it had greater similarity to a diploid than to a tetraploid spike. It was six-rowed with well developed lateral florets and rough awns. On the average, 36 to 40 spikelets per head were produced. The internodes of the spike were short and the spike was only half as long as a normal diploid spike. The awns seemed to be sturdier than those of a diploid but they were short and not typical of the O.A.C. 21 variety. The flowering glumes were the same size as those of diploid O.A.C. 21 and opened during anthesis. The anthers were very small, partly shrunken, and usually did not dehisce.

To date three tillers have been studied and these failed to produce seed. After flowering glumes opened there seemed to be some swelling in the female parts of the flowers, giving an impression that fertilization might have taken place. Therefore, some of the florets were removed at this stage and placed on Orchid agar medium in glass vials. No further growth, however, was noted.

TABLE 1.—CHROMOSOME NUMBER AND FERTILITY OF THE DISCUSSED  $C_4$  PLANTS

Plant No.	Number of chromosomes	Per cent fertility
322	28	83.3
323	28	0 (dwarf)
324	29	10.0
325	27	0 (dwarf)
326	19	0

TABLE 2.—CHROMOSOME ASSOCIATIONS OF THE 19-CHROMOSOME BARLEY PLANT AT FIRST METAPHASE

Type of configuration	Frequency	
	No.	Per cent of total
5 III + 2 II	26	8.7
4 III + 3 II + 1 I	109	36.3
3 III + 4 II + 2 I	103	34.3
2 III + 5 II + 3 I	50	16.7
1 III + 6 II + 4 I	9	3.0
7 II + 5 I	3	1.0
Total	300	100.0

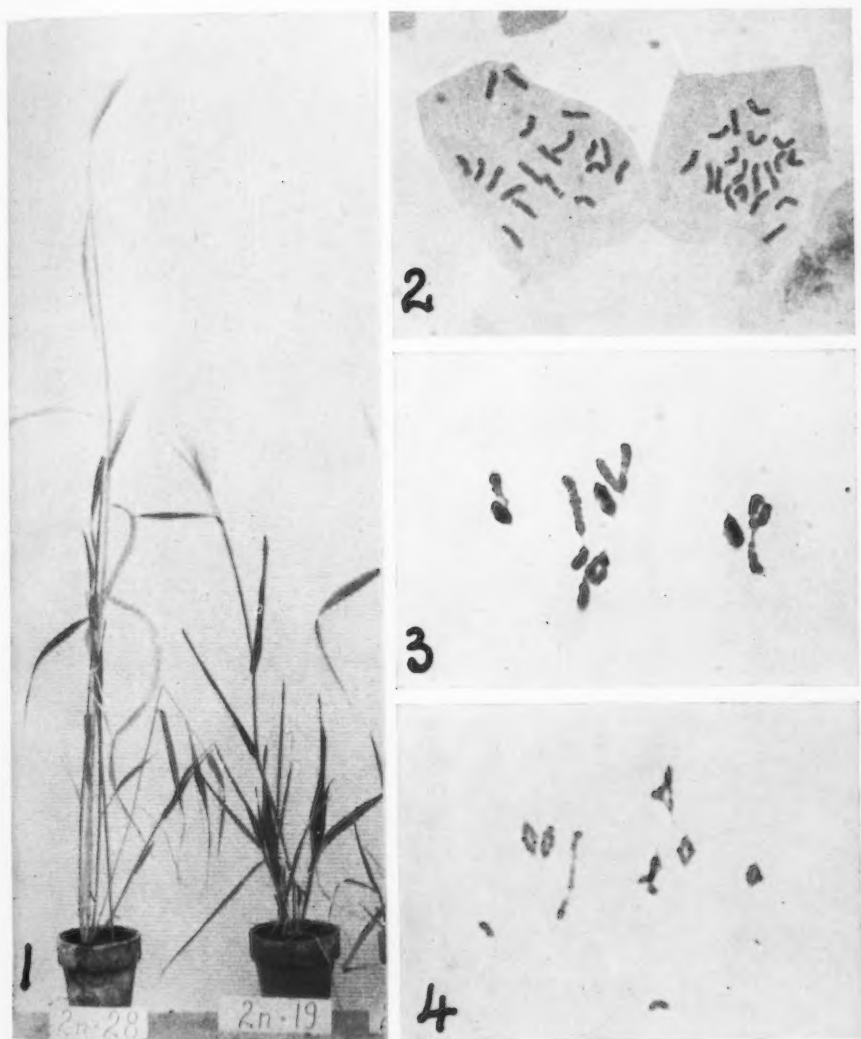
The observed fertility of the siblings obtained from the same parent as the 19-chromosome plant varied from 0 to 83 per cent and the data are given in Table 1. These four plants did not differ in morphology from the other O.A.C. 21 plants with similar deviations in chromosome number.

#### CYTOLOGICAL OBSERVATIONS

Chromosome counts were first made when the plant was in the two-leaf stage. The aceto-carmin squash method was used to determine the chromosome number in root tips. Nineteen chromosomes were found in every cell examined (Plate I, Figure 2). Chromosome counts in root tips were made several times during the development of the plant and no changes were observed. The four siblings of the 19-chromosome plant had chromosome numbers ranging from 27 to 29 (Table 1).

Three hundred PMC's from the 19-chromosome plant were examined at first meiotic division for chromosome associations using the aceto-carmin squash technique. No quadrivalent associations were observed. The results are presented in Table 2. It can be seen that the number of univalents and trivalents fluctuated from zero to five and the number of bivalents from two to seven. Seventy per cent of the PMC's examined showed chromosome associations of 4 III + 3 II + 1 I or 3 III + 4 II + 2 I.





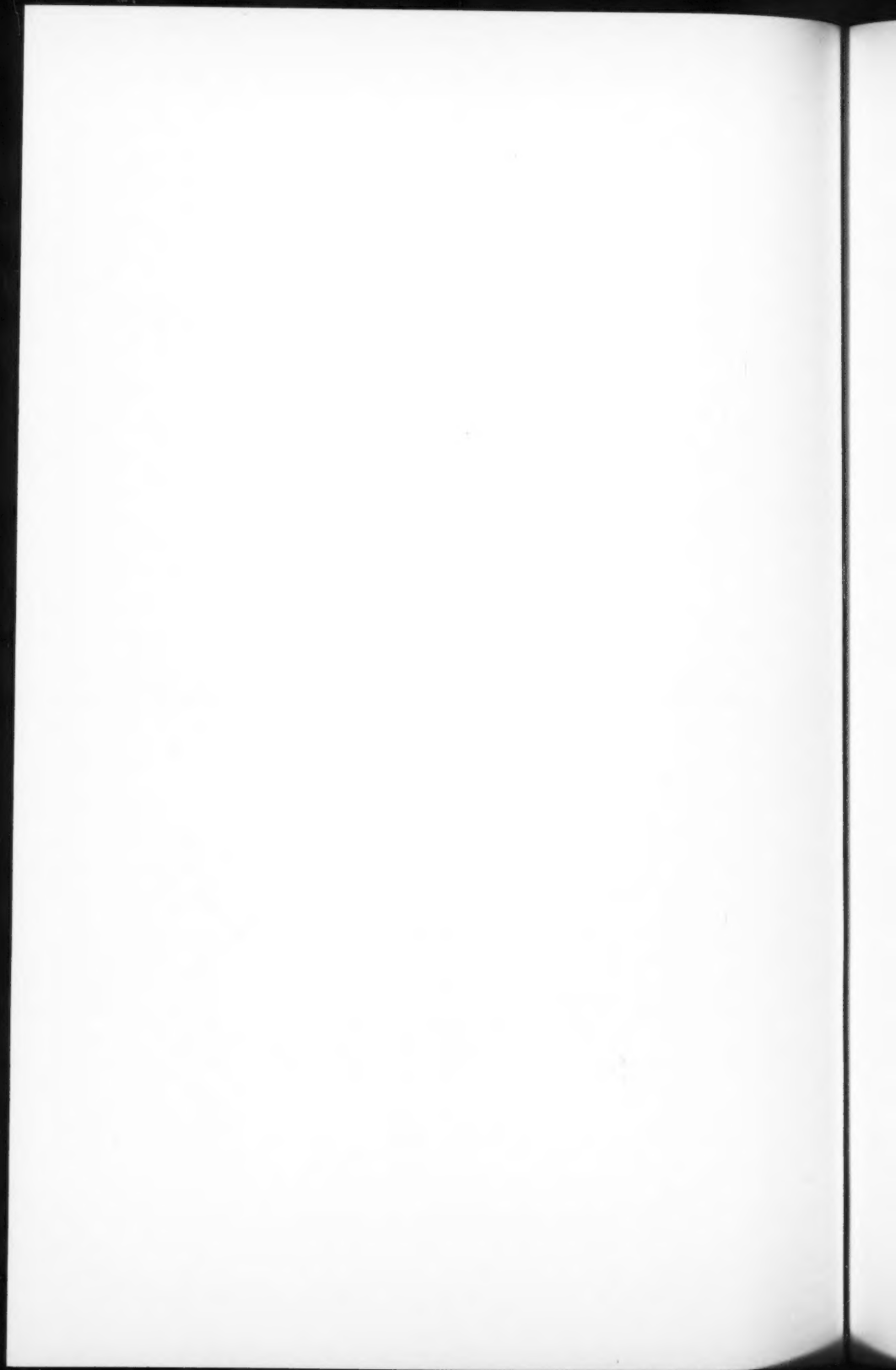
# PLATE I

FIGURE 1.—O.A.C. 21 barley plants, with 28 and 19 chromosomes. (ca. 1/9 X).

FIGURE 2.—Mitotic metaphases showing 19 chromosomes. (ca. 700 X).

FIGURE 3.—First meiotic metaphase, 5 III + 2 II (ca. 700X).

FIGURE 4.—First meiotic metaphase, 3 III + 4 II + 2 I. (ca. 500 X).



The bivalent associations were as complete as those observed in a normal diploid barley. The trivalents were also closely associated and formed figures in the shape of a V, a Y, a "frying-pan" or a chain (Plate I, Figures 3 and 4). In the chain of three, the third chromosome seemed to be only loosely attached to the others.

In general, no abnormalities were observed during the meiotic process. At anaphase I, the chromosomes associated in trivalents disjoined regularly, i.e., two went to one pole and one to the other pole. No laggards were observed and the division in second anaphase was normal.

Observations of pollen mitosis showed that the pollen grains contained 8 to 12 chromosomes. Most of them, however, had only one nucleus and very little cytoplasm and many were completely abortive.

The meiotic analysis showed only simple association of trivalents, bivalents and univalents as presented in Table 2. These findings indicate that the 19-chromosome plant has 2 complete basic chromosome sets, and, in addition, five chromosomes which show no homology with one another, i.e.  $2(abcdefg) + (abcde) = 19$ .

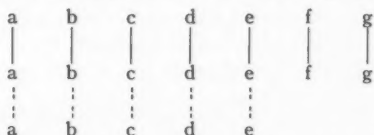
#### DISCUSSION

The lowest chromosome number previously reported in basically tetraploid barley is 24 (4). This indicates that gametes with less than 10 chromosomes, even on the female side, apparently do not survive. Therefore, the occurrence of the 19-chromosome plant by a chance union of deficient diploid gametes is not very likely.

The 19-chromosome plant could also have arisen from the union of haploid gametes possessing extra chromosomes. However, in this case these gametes should have at least 2 to 3 chromosomes more than the normal haploid gametes. Such gametes usually are inviable in a diploid plant.

The cytological data suggest another explanation. The meiotic configurations listed in Table 2 indicate the presence of 2 complete genomes and 5 additional chromosomes. Because quadrivalents were not found, the 5 additional chromosomes apparently are not homologous to each other. In such a case, the chromosome constitution of the 19-chromosome plant could be illustrated as shown in Diagram 1. In this diagram, the unbroken line indicates a constant association between homologous chromosomes. The broken line indicates only occasional associations causing trivalents or univalents.

DIAGRAM 1.—PROPOSED CHROMOSOME ASSOCIATION IN THE 19-CHROMOSOME PLANT



Such constitution could be obtained in a plant which has arisen from the union of a  $2n-2$  gamete with a normal haploid gamete, i.e.,  $12 + 7$ .

Our data, and data in the literature, indicate that  $2n-2$  chromosome gametes are functional in tetraploid barleys.

The deviations in chromosome number among siblings of the 19-chromosome plant show that deficient gametes were produced by the parent plant. As the seed parent was growing close to a row of diploid O.A.C. 21 barley, it seems very likely that the 19-chromosome plant was produced by the union of a normal haploid gamete from the diploid variety with a 12-chromosome egg from the tetraploid.

#### REFERENCES

1. Chen, Shao-lin, Shu-min Shen, and P. S. Tang. Studies on colchicine-induced auto-tetraploid barley. I and II. Cytological and morphological observations. *Amer. J. Botany* 32:103-106. 1945.
2. Dorsey, E. Chromosome doubling in the cereals. *J. Heredity* 30:393-395. 1939.
3. Müntzing, A. Experiences from work with induced polyploids in cereals. *In Svalof 1886-1946*, pp. 324-337. Carl Bloms Boktryckeri A.-B., Lund. 1948.
4. Rosendahl, G. Cytologische Untersuchungen an Tetraploiden Gersten. *Kühn-Arch.* 60:238-252. 1944.

# STUDIES ON THE CYTOLOGY OF BROMEGRASS, *BROMUS INERMIS* LEYSS.<sup>1</sup>

BJÖRN SIGURBJÖRNSSON<sup>2</sup>, AKIRA MOCHIZUKI<sup>3</sup> AND JOHN D. TRUSCOTT<sup>4</sup>

University of Manitoba, Winnipeg, Manitoba

[Received for publication June 10, 1957]

## ABSTRACT

A population of 222 plants of 16 varieties and strains of northern and southern brome grass was studied for somatic chromosome number. The following observations were noted: Aneuploid plants, including the chromosome numbers 54, 55, 57 and 58, were found within the species, *Bromus inermis*. One plant was found to have 49 chromosomes. A cytological difference was found between certain varieties of northern and southern types of brome grass. The northern type had a significantly higher number of aneuploid plants with chromosome numbers in excess of 56, while the southern type had more aneuploid plants with fewer than 56 chromosomes. Some evidence was found suggesting that selection of northern type strains may bring about a simultaneous selection for additional chromosomes, whereas selection for southern type strains may involve a simultaneous selection for plants with missing chromosomes. Average seed set was found to be lowered in the presence of two additional chromosomes or absence of two chromosomes from the euploid complement.

## INTRODUCTION

Brome grass, *Bromus inermis* Leyss., is considered one of the most important forage species in North America. It is a long-lived, perennial, cross-pollinated plant with strong creeping rhizomes which form a dense and heavy sod with an abundance of vegetative growth. It was introduced into this continent in the late nineteenth century and since has been grown widely throughout Canada and the United States, especially in regions of moderate rainfall and low-to-moderate summer temperatures.

Zherebina (15) divided brome grass into two distinct types, the "meadow" type and the "steppe" type, which he described as the sub-species *borealis* and *australis* respectively. The two sub-species differed in their areas of distribution, in various minor morphological characters and in susceptibility to certain diseases.

Newell and Keim (10) reported on similar differentiation of brome grass types in the United States. They termed the types "northern" and "southern" and these types were found to correspond reasonably well to the "meadow" and "steppe" types respectively, as described by Zherebina. Knowles and White (8) concurred in this division of the species. The northern type was introduced to North America from Siberia and is well adapted to Canada and the Northern Great Plains. The "southern type" was introduced from central Europe and is particularly well adapted to the Corn Belt States and some parts of the central Great Plains.

Much of the seed supply for Canada and the United States is produced from the northern type brome grass in the Prairie regions of Canada. Because the northern type is not so well adapted to the southern regions

<sup>1</sup>Portion of thesis presented by the senior author to the Faculty of Graduate Studies, University of Manitoba, in partial fulfillment of requirements for the M.Sc. degree.

<sup>2</sup>Graduate Student.

<sup>3</sup>Post-Doctorate Fellow.

<sup>4</sup>Associate Professor.

as the southern type, plant breeding programs are under way for the purpose of breeding varieties of brome grass that will give high seed yields in northern regions, and yet produce satisfactory yields of forage in southern regions. Programs of this sort may require the use of plants of both the southern and northern types. The present study includes a cytological survey of a number of varieties and strains of both these types.

Somatic chromosome numbers for *B. inermis* have been reported by many workers. Avdulov (2) reported a somatic count of  $2n=56$  from brome grass. This number was also observed by other investigators (5, 7, 9, 11). A chromosome complement of  $2n=42$  was first noted by Stahlin (14) and subsequently confirmed independently by Knobloch (7), Nielsen (11) and Atwood (1). Chromosome complements of  $2n=70$  and  $2n=28$  have also been observed (11, 12). Schulz-Schaeffer (13) counted 62 chromosomes in his brome grass material in 1956. He found two types of chromosomes in the haploid complement, 21 of one type and 10 of the other. The second type possibly were inert B-chromosomes. Hill and Myers in 1948 (6) reported on chromosome numbers in 163 plants of brome grass. They found 159 plants with 56 chromosomes, 3 with approximately 56, and 1 with the normal complement of 56 chromosomes plus 0 to 11 fragments, which they thought were analogous to inert B-chromosomes. In their discussion Hill and Myers stated that "the possibility of aneuploid plants cannot be excluded". Darlington and Wylie in their "Chromosome Atlas 1954" (3) list the following numbers for the species: 28, 42, 56,  $56+0-11f$ , 70.

#### MATERIALS AND METHODS

The survey of somatic chromosome numbers consisted of two phases, a preliminary survey involving a few plants of each of a number of varieties and strains of the two types, and a second survey involving a larger number of plants from two northern and two southern types.

The plants had been space-planted in the experimental fields at the University of Manitoba in the spring of 1954. Root cuttings were transferred to 4-inch pots in the Plant Science greenhouse during the fall of the same year and the preliminary chromosome survey was made the following winter.

When the plants had become well established, root tips were collected, placed in water in small glass vials and left in a refrigerator overnight. Farmer's solution (3 parts 95% ethyl alcohol: 1 glacial acetic acid) was used for fixation, the root tips being left in the fixative for at least 3 hours. The root tips were then washed in water and hydrolysed in 1N hydrochloric acid at 60° C. for 7-10 minutes. The root tips were stained in a leucobasic fuchsin solution, (Feulgen), and squashed in 45 per cent acetic acid.

Spreading of the chromosomes was accomplished by heating and tapping on the coverslip intermittently. At least three well spread metaphases were counted for each determination, and up to forty, if the chromosome complement was suspected of being incomplete.

After the preliminary survey, a second survey was conducted in order to establish the range in chromosome numbers and test the stability of the chromosome complement of the variety Southland.



The cytological procedures employed in the second survey were identical to the procedures described above, and the source of materials was the same field of spaced plants utilized in the preliminary survey. Cytological differences between the northern and southern types of brome grass were evaluated by the chi-square test as outlined by Dixon and Massey (4).

### RESULTS AND DISCUSSION

In the preliminary survey, somatic chromosome counts of 107 plants were made, 59 plants of 6 varieties and strains of the northern type, and 48 plants of 10 varieties and strains of the southern type. Table I reports the chromosome numbers found in the survey and their distribution.

TABLE I.—CLASSIFICATION OF PLANTS ACCORDING TO THEIR CHROMOSOME NUMBERS

Varieties	Somatic Chromosome Numbers					Total
	54	55	56	57	58	
Canadian Comm.		1	7	2		10
53-7			5	4	1	10
175-44		1	5	5		11
216-20		2	5	1	2	10
175-29		2	6			8
194-12		1	9			10
<i>Total Northern</i>		7	37	12	3	59
Southland			5			5
Fischer	1		3	1		5
Lancaster			4			4
Lyon			2	1	1	4
567			6			6
561		1	3	1		5
383	1	1	2		1	5
BR-3	1	2	2			5
510			4			4
475		1	4			5
<i>Total Southern</i>	3	5	35	3	2	48
Grand Total	3	12	72	15	5	107

For the second survey, the varieties Canadian Commercial and 53-7 of the northern type, and Southland and Fischer of the southern type were selected. A total of 145 plants were investigated, 31 of Canadian Commercial, 32 of 53-7, 36 of Southland, and 46 of Fischer. The results of this survey are shown in Table 2.

Table 3 shows the total distribution of chromosome numbers of 222 plants within the southern and northern types of brome grass when both surveys are combined.

The preliminary survey revealed a rather high occurrence of aneuploid chromosome numbers in the species *Bromus inermis*. Out of a total of 107 plants 35, or 32.71 per cent were found to be aneuploid.

The chromosome numbers found in the preliminary survey for this species are: 54, 55, 56, 57 and 58, of which all but 56 are reported for the

TABLE 2.—CLASSIFICATION OF PLANTS ACCORDING TO THEIR CHROMOSOME NUMBERS\*  
(Second Survey)

Varieties	Somatic Chromosome Numbers						Total
	49	54	55	56	57	58	
Canadian Comm. 53-7		1	5 1	19 22	5 7	1 2	31 32
<i>Total Northern</i>		1	6	41	12	3	63
Southland Fischer	1	4 1	6 11	24 26	2 6	1	36 46
<i>Total Southern</i>	1	5	17	50	8	1	82
Grand Total	1	6	23	91	20	4	145

\*Plants from the same 4 varieties studied in the preliminary survey are included in this classification

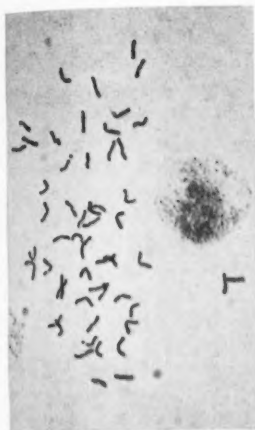
TABLE 3.—CLASSIFICATION OF ECOTYPES ACCORDING TO THEIR CHROMOSOME NUMBERS  
(RESULTS OF THE PRELIMINARY AND THE SECOND SURVEY COMBINED)

Ecotypes	Somatic Chromosome Numbers						Total
	49	54	55	56	57	58	
Northern		1	12	66	18	5	102
Southern	1	7	22	77	10	3	120
Total	1	8	34	143	28	8	222

first time (see Plate I, A, B, C, D and E). Some of the varieties studied in this survey, however, did not show any aneuploidy. For example, 5 plants of the variety Southland had the chromosome number 56. This variety, along with 3 other strains which showed a fair amount of variation in the preliminary survey, were subjected to a more extensive study in the second survey.

In this second survey, 31 plants of the variety Southland showed a considerable variation in chromosome numbers, ranging from 54 to 57. The other varieties also showed a similar distribution of chromosome numbers, as reported in Table 3. Thorough testing of one plant of the Fischer variety confirmed its somatic chromosome complement to be 49 (see Plate I, F). This chromosome number has not been reported previously for bromegrass and it is still doubtful whether this plant is a true aneuploid with 7 missing chromosomes or whether it is euploid and represents a cross between hexaploid, 42-chromosome, and octoploid, 56 chromosome, bromegrass.

Preliminary studies in this material show that irregularity at meiosis is considerable. This phase of the study has just commenced and will be reported at a later date. Aneuploidy, whether it represents additional or



A. Mitotic Metaphase  
54 chromosomes  
(X1200)



B. Mitotic Metaphase  
55 chromosomes  
(X1600)



C. Mitotic Metaphase  
56 chromosomes  
(X1600)



D. Mitotic Metaphase  
57 chromosomes  
(X1100)



E. Mitotic Metaphase  
58 chromosomes  
(X1100)



F. Mitotic Metaphase  
49 chromosomes  
(X1600)

PLATE 1



missing chromosomes, usually has a disturbing effect on plants, especially when arising from diploids. Meiotic irregularity often has profound effects upon the fertility and reproduction of a species so as to limit its competence and distribution. The aneuploidy found in the plants studied may be related to the irregularity at meiosis which the preliminary studies indicate.

Brome grass, being one of the higher polyploids, could behave quite normally in spite of presence or absence of 1 or 2 chromosomes. The aneuploid plants, along with the 49 chromosome plant, were found to compare favourably with the euploid plants in leaf production and rhizome vigour. The aneuploids having either 2 missing or 2 additional chromosomes and the 49-chromosome plant were found to be lower in average seed-setting ability. The 55 and 57 chromosome plants were comparable to the 56 chromosome plants in seed-setting ability, as shown in Table 4.

The meiotic irregularity may not interfere to any great extent with the vegetative development and spreading rate of brome grass. It is a long-lived perennial, extensively spreading by vigorous rhizomes and is not dependent exclusively on sexual reproduction for its distribution. Elliott and Love (5) postulated that an exceptionally vigorous clone may advance and spread extensively far beyond a normal individual of the species even though, because of irregularities at meiosis and/or disturbances caused by aneuploidy, it may rarely or never have produced seeds during its lifetime.

In the analysis of the aneuploidy found in this study, results from the two surveys were pooled as shown in Table 3 and studied together. Because of the unknown origin of the 49-chromosome plant, it is not included in this analysis. The total aneuploidy within the 221 plants studied was found to be evenly distributed among the two types, southern and northern brome grass, as shown in Table 5.

TABLE 4.—FERTILITY RATIO VS. CHROMOSOME NUMBERS

Chromosome No.	Number of plants	Fertility range	Ratio* mean
49	1	—	.067
54	6	.027 — .557	.342
55	23	.068 — .636	.450
56	91	.009 — .692	.470
57	27	.328 — .664	.513
58	4	.105 — .503	.303

\*Ratio of cleaned seed weight to weight of unthreshed panicle

TABLE 5.—ANALYSIS OF THE FREQUENCY OF ANEUPLOID PLANTS WITH RESPECT TO ECOTYPES

Ecotypes	Number of Plants		Total	Percentage
	Euploid	Aneuploid		
Northern	66	36	102	35.29
Southern	77	42	119	35.29
Total	143	78	221	35.29

TABLE 6.—ANALYSIS OF THE DISTRIBUTION OF TYPES OF ANEUPLOIDY WITH RESPECT TO ECOTYPES

Ecotypes	Aneuploidy				
	Total Plants	Number <56	Per cent <56	Number >56	Per cent >56
Northern	36	13	36.11	23	63.89
Southern	42	29	69.05	13	30.95
Total	78	42	53.85	36	46.15

The data were then rearranged to test whether the types differed with respect to the kind of aneuploidy present. Since the portion of aneuploidy was found to be equal within each of the northern and southern types, this feature can be examined directly without consideration of the euploid plants. The percentage of classified aneuploidy shown in Table 6 is, therefore, based on the number of aneuploid plants within each type only.

It is evident that the varieties of the two types studied differ with respect to kind of aneuploidy, the northern exhibiting a higher frequency of plants having chromosome numbers in excess of 56, the southern showing the reverse situation. This difference was tested by using the statistic "chi-square" and was found to be significant at the 1 per cent level. This cytological difference between the two types has not been reported previously, according to available literature.

This study has not indicated whether plants having the additional chromosomes conform more specifically to the northern type of brome grass than do plants with 56 chromosomes or lower, or whether plants lacking 1 or 2 chromosomes conform more closely to the concept of the southern type. It is of interest, however, to refer to Table 2 and view the distribution of chromosome numbers of the 4 varieties investigated in the second survey. The Canadian Commercial variety is a very heterogeneous population of plants which cannot easily be identified as belonging to one variety. It may be termed the "wild type" of northern brome grass, consisting of a number of types of plants. The strain 53-7 is an open-pollinated selection from the variety Superior which, in turn, is a selection from Canadian Commercial. Both Superior and 53-7 were selected as northern type strains, tested and selected in areas where northern types are better adapted. Thus the selection of 53-7 was presumably based on characters typically northern, representing the northern characteristics of the commercial wild type. The aneuploid distribution of the Canadian Commercial variety appears symmetrical in Table 2. The variety 53-7 is very different. Only one plant in 32 had a chromosome number lower than 56 but 9 had chromosome numbers in excess of 56. Whether 53-7 conforms more closely to the northern type of brome grass than the commercial variety is not known, and no further attempt to substantiate this finding was made in this study. The question may be posed, but not answered, whether selection within the Canadian Commercial brome is tending toward selection of aneuploid plants



having a somatic chromosome number in excess of 56. The relationship between the 2 southern varieties appears to be similar though not as distinct. The data on southern varieties reported in Table 2 indicates a trend in the opposite direction, that is, toward a somatic chromosome number of less than 56.

#### ACKNOWLEDGEMENTS

This study was made possible by the financial assistance of the National Research Council of Canada. The senior author wishes to express his gratitude to the National Research Council for the Bursary which supported this investigation.

#### REFERENCES

1. Atwood, S. S. Cytogenetics and breeding of forage crops. Advances in genetics 1, pp.1-67. Academic Press Inc., New York, N.Y. 1947.
2. Avdulov, N. P. Karyo-systematische Untersuchung der Familie Gramineen. Bull. Appl. Botan. Genetics and Plant Br., Suppl. Vol. 44:1-428. 1931.
3. Darlington, C. D., and A. P. Wylie. Chromosome atlas of flowering plants. G. Allen & Unwin Ltd., London, England. 1955.
4. Dixon, W. J., and F. J. Massey. Introduction to statistical analysis. McGraw-Hill Book Co., Inc., New York, N.Y. 1951.
5. Elliott, F. C., and R. M. Love. The significance of meiotic chromosome behavior in breeding smooth brome grass, *B. inermis* Leyss. J. Amer. Soc. Agron. 40:335-341. 1948.
6. Hill, H. D., and W. M. Myers. Chromosome number in *Bromus inermis* Leyss. J. Amer. Soc. Agron. 40:466-469. 1948.
7. Knobloch, I. W. Morphological variations and cytology of *Bromus inermis*. Bull. Torrey Botan. Club 70:467-474. 1943.
8. Knowles, R. P., and W. T. White. The performance of southern strains of brome grass in Western Canada. Sci. Agr. 29:437-450. 1949.
9. Love, Askell, and Doris Love. Cytotaxonomical studies on boreal plants. III. Some new chromosome numbers of Scandinavian plants. Arch. Botanik 31:1-23. 1944.
10. Newell, L. C., and F. D. Keim. Field performance of brome grass strains from different regional sources. J. Amer. Soc. Agron. 35:420-434. 1943.
11. Nielsen, E. L. Grass studies. III. Additional chromosome complements. Amer. J. Botany 26:366-371. 1939.
12. Nielsen, E. L. Cytology and breeding behavior of twin plants of *B. inermis*. Botan. Gaz. 113:23-54. 1951.
13. Schulz-Schaeffer, J. Cytologische Untersuchungen in der Gattung *Bromus* Leyss. Zeitschrift für Pflanzenzuchtung 35:297-320. 1956.
14. Stahlin, A. Morphologische und zytologische Untersuchungen an Gramineen. Wissenschaftliches Archiv. F. Landwirtsch. Abt. A. Pflanzenbau. 1:330-398. 1929.
15. Zhrebina, Z. N. Botanical-agronomical studies of awnless brome grass (*Bromus inermis* Leyss.) Bull. Appl. Botan. Genetics and Plant Br. 25:201-352. 1931.

## NOTE ON AN AUTOMATIC START DEVICE FOR FROST CONTROL BY SPRINKLER IRRIGATION

Late spring and early fall frosts constitute a major hazard in the production of horticultural crops. Protecting strawberries, tomatoes and other low-growing fruit and vegetable crops from frost is economically attractive because of the greater potential market. One method that will decrease frost damage is by water application through sprinklers.

It has been reported by Davis (1) that crops have been protected against temperatures as low as 20° F. Protection to this low temperature cannot be achieved if heat is lost because of cold winds.

For general frost control, standard sprinkler irrigation equipment can be used. Some type of temperature alarm should be used so that, when a critical temperature is indicated, the system may be started manually. In experimental work it is desirable to have an automatic control device to turn the water on at the critical temperature. Such a system was developed at the Ontario Agricultural College in 1956 and tested in the spring of 1957.

The equipment used to automatically start the application of water was a 110-volt motorized valve assembly and a temperature controller\*. A T415A remote bulb thermostat was located close to the field experiment and placed 1 foot above the ground level, as shown in Figure 1. The temperature controller, by means of a single pole single throw mercury switch, closed an electrical circuit when the temperature fell to 34° F.

The controller actuates a M204A Modutrol Motor-Series 20 which is uni-directional. A Q601A Valve Linkage adapts the motor to a V537E Valve Body. An external indicator shows the position of the valve when in operation. The valve lift is adjustable and a strain release mechanism is built into the linkage to ensure tight seating of the valve disk. The motorized valve assembly as shown in Figure 2 was located 200 feet from the temperature controller in the field. The valve size is 2-inch and the time of opening, which is not critical, is 60 seconds. The valve, which opens automatically, remains open until closed manually by a push-button. This is an advantage in frost control where the system must be kept in operation until all the ice has been melted from the plants. The entire assembly costs less than \$250.00.

Three varieties of tomato plants were field set, May 14, 1957. On May 16, the recorded temperature in the field was 34° F. at 1.00 a.m. and 30.5° F. at 6.00 a.m. In the test the total plant survival was 89 per cent, with the only loss occurring in the area of a plugged sprinkler.

This device has proved to be satisfactory for automatically starting a sprinkler irrigation system at a critical temperature. The human factor of failing to rise when the temperature alarm sounds is eliminated. The automatic start device may thus result in the saving of a valuable experiment.

\*Minneapolis-Honeywell Regulator Co., Ltd., Toronto, Ont.



FIGURE 1. Temperature controller and remote bulb thermostat.



FIGURE 2. Motorized valve assembly.

REFERENCE

- (1) Davis, J. R. Frost protection with sprinkler irrigation. Mich. Agr. Expt. Sta. Extension Bull. 327, 1955.

—R. W. IRWIN,  
Department of Engineering Science,  
Ontario Agricultural College, Guelph, Ont.

—H. TIESSEN,  
Department of Horticulture,  
Ontario Agricultural College, Guelph, Ont.

October 7, 1957

## NOTE ON A FLUORESCENCE TEST TO DISTINGUISH SEEDS OF ALFALFA AND SWEET CLOVER IN MIXTURES

Sweet clover (*Melilotus alba* and *M. officinalis*) and alfalfa (*Medicago sativa*) often occur together in commercial seed. Competent seed analysts, using small differences in morphology, can make separations required for seed purity tests, but the work is exacting. For the seedsman, and possibly for the seed analyst, a simple physical or chemical test which would distinguish the two kinds of seed would be useful.

It is thought that a basis for a simple test to distinguish sweet clover and alfalfa seeds might be in the well-known fact that the former contains coumarin while the latter does not. Colour reactions with coumarin did not offer a simple enough test. It was found, however, that coumarin relatives and possibly other compounds in alfalfa fluoresced brilliantly under ultra-violet light at pH levels around neutrality. Coumarins in sweet clover did not fluoresce without alkali treatment.

Fluorescence tests with other seeds are referred to by Radley (1). In each of our tests, 25 seeds were spaced on moist Whatman No. 1 filter paper in Petri plates *ca.* 9 cm. in diameter. Hydrolytic processes were sufficiently advanced after a few hours to permit a first reading under ultra-violet light. Readings were continued over a 96-hour period. Slowly germinating or hard seeds, which do not imbibe moisture, were not included in the reading. Scarification of seed shortened the time in which readings could be made and increased the number of seeds available for each test. Several sources of ultra-violet light were used and all were satisfactory. Alfalfa fluorescence was not dissipated by drying, heating to 105° C. for one hour, light washing with water, or germination on glass or cellophane.

Germinating alfalfa seeds of various ages and sources from the following species, varieties, and strains fluoresced: *Medicago ruthenica*, *M. glutinosa*, *M. falcata* vars. F. C. 23631, Don, and Oregon Creeper, *M. sativa* vars. Cossack, Ladak, Rhizoma, Grimm 666, Grimm Vidarshov, Nemastan Ranger, Talent, Nomad, Sevelvra, Atlantic, and Vernal. The annual alfalfas, *M. lupulina*, *M. hispida*, and *M. tribuloides*, give a root tip fluorescence rather like that of *Trifolium* spp. No fluorescence occurred with germinating seeds of Madrid, Evergreen, Albotrea, or Hubam sweet clover varieties; with commercial *Melilotus alba* and *M. officinalis*; with *M. suaveolens*, *M. dentata*; or with coumarin-free sweet clover obtained from Ross Greenshields, of the Canada Department of Agriculture Forage Laboratory, Saskatoon, Saskatchewan.

In our experience, all germinating alfalfa seeds have fluoresced brilliantly and all sweet clover seeds have failed to fluoresce on filter paper. In view of the variations known to occur in other luminescence tests, and in view of observations such as those of Trumble and Phipps (2), viz., that *Trifolium subterraneum* seeds from South Australia did not fluoresce, but that those from West Australia did, it seems prudent to suggest that seedsmen and seed analysts look critically at this test for distinguishing alfalfa and sweet clover seed. Work is proceeding to characterize the fluorescent compounds of germinating alfalfa.



The estrogen, coumestrol, isolated and characterized by Bickoff, E. M., *et al.*, Science 126: 969-970, November 8, 1957, appears to be the principal compound responsible for fluorescence in *Medicago sativa* seeds.

#### REFERENCES

1. Radley, J. A., and J. Grant 1954. Fluorescence analysis in ultra-violet light. 4th ed. Chapman and Hall Ltd., London, England.
2. Trumble, H. C., and I. F. Phipps, 1933 The inheritance of fluorescence in hybrids between perennial rye-grass and wimmera rye-grass. J. Australia, Council Sci. Ind. Res. 6:170-178.

—V. C. BRINK,  
Division of Plant Science,  
The University of British Columbia,  
Vancouver 8, B.C.

April 12, 1957

# NOTE ON THE EFFECT OF PHOTOPERIOD AND TEMPERATURE ON THE DEVELOPMENT OF SPIKE PRIMORDIA IN BARLEY

Interest in earliness in barley as a genetic character led to the present study. Genetically, earliness has been shown to be a quantitative character, the expression of which is determined by interaction between the genotype and environment involved. Among such environmental factors, the most important are probably photoperiod and temperature. Consequently, an experiment was designed to show the influence of these factors on the development of spike primordia, which is directly related to the rate of maturity.

Seedlings of a group of spring barley varieties, ranging from early to late maturity, were grown in flats placed in three greenhouse sections which were held at temperatures of 55°, 65° and 75° F., respectively. At

TABLE 1.—SPIKE-PRIMORDIUM LENGTHS IN MILLIMETRES OF BARLEY VARIETIES AFTER 12, 29 AND 43 DAYS' GROWTH UNDER CONTROLLED CONDITIONS OF LIGHT AND TEMPERATURE (F.)

Variety	Stage, days	13-hr. exposure			17-hr. exposure		
		75°	65°	55°	75°	65°	55°
Atsel (D.T.H. = 48)*	12	0.3	0.2	—	0.8	0.5	—
	29	1.6	1.7	1.2	4.0	4.0	2.0
	43	2.5	2.0	1.5	16.0	6.0	3.0
Tulare (D.T.H. = 49)	12	0.2	—	—	0.4	0.3	—
	29	1.2	1.7	1.0	3.5	2.7	2.0
	43	1.3	3.0	1.8	5.0	4.0	3.0
Beecher (D.T.H. = 51)	12	—	—	—	—	—	—
	29	1.5	1.5	1.2	3.0	2.0	1.5
	43	1.8	2.0	1.5	4.0	3.5	2.5
Gateway (D.T.H. = 53)	12	—	0.1	—	—	—	—
	29	1.5	1.4	1.5	3.0	3.0	2.0
	43	1.6	2.0	1.4	3.5	3.0	1.5
Titan (D.T.H. = 56)	12	—	—	—	0.3	0.2	—
	29	1.2	1.0	1.0	2.0	2.0	1.2
	43	1.5	1.7	1.5	3.5	2.5	1.5
Newal (D.T.H. = 59)	12	—	—	—	—	—	—
	29	1.5	1.2	1.0	2.0	2.0	1.5
	43	1.4	1.8	1.2	3.5	4.5	2.0
Montcalm (D.T.H. = 61)	12	0.2	—	—	0.5	0.2	—
	29	1.2	0.2	0.8	1.5	2.5	1.6
	43	1.2	1.2	1.3	4.0	2.5	2.0
Bonneville (D.T.H. = 63)	12	—	—	—	—	—	—
	29	1.5	0.3	0.2	1.5	1.8	1.5
	43	1.2	1.3	1.2	2.5	2.0	1.4
Frontier (D.T.H. = 66)	12	—	—	—	—	—	—
	29	1.0	0.2	0.5	2.0	1.0	1.7
	43	1.3	1.0	1.0	2.5	1.3	1.3

\*D.T.H.—Days to heading, the average time elapsed from sowing to emergence of the first spikelet above the flagleaf, under optimum field conditions.

each temperature a short and a long photoperiod (13 and 17 hours) was obtained by controlling incident lighting by a system of electric lamps and shades.

A typical seedling of each variety was collected for a given determination, washed, killed for 48 hours in Pharmer's Mixture (3 parts of 95 per cent ethanol to 1 part of glacial acetic acid), and washed and stored in 70 per cent ethanol. Primordia were dissected and measured under a microscope.

Length measurements of spike primordia, taken 12, 29 and 43 days after sowing from each photoperiod-temperature condition, are given in Table 1. Determinations made on seedlings 9 and 16 days after sowings (not included in the Table) together with the 12-day stage, showed that spike-primordium initiation coincided with the appearance of the fifth leaf.

The data in Table 1 show that photoperiod is a much more important factor than temperature in primordial development. With 13 hours' exposure, the effect of temperature in each of the later stages is, with rare exception, negligible. With 17 hours' exposure the effect of temperature is marked, especially in the earlier varieties. Here temperature reflects the effect of accelerated development under conditions of greater warmth, a phenomenon almost wholly suppressed at the shorter photoperiod. The most significant point to the Table, therefore, is the relatively great differences in primordial lengths as between the 17- and 13-hour photoperiods.

This information cannot be obtained from sowing at various dates in the field, because the seasonal increase in photoperiod is inextricably associated with seasonal increase in mean temperature.

Translating the significance of these results in terms of both genetic and agronomic earliness studies, the conclusion must be that photoperiod is probably the basic factor determining the expression of earliness in spring barleys, a point which must be taken into account when comparing data from material sown at different times or grown in different latitudes.

—L. P. V. JOHNSON,  
Department of Plant Science,  
University of Alberta,  
Edmonton, Alberta

—A. R. TAYLOR,  
Box 71,  
Khartoum North,  
Sudan

November 19, 1957

## NOTE ON AN UNUSUAL TROPISM OF FEEDER ROOTS IN SUGAR BEETS AND ITS POSSIBLE EFFECT ON FERTILIZER RESPONSE

The pattern of growth of sugar beet roots at a number of locations in the Red River Valley of Manitoba was studied over a period of many years by the author. One observation of interest was that the two creases (Figure 1) and associated feeder roots of sugar beets were always orientated in an east/west direction respectively. The cause of this tropism is being studied.

Anatomically, the root system of the sugar beet can be divided into three zones in which roots differ in shape and position in the soil. The *First Zone* consists of the upper part of the main root to a depth of approximately 2 inches. Growing out of the creases are masses of very fine feeder roots orientated in an east/west direction. Growing out of the *Second Zone* of the main root (2 to 6 inches in depth) are stronger developed, widely spreading side roots with feeder roots, both also orientated in an east/west direction. The *Third Zone* or tail of the root carries a far less amount of side roots and very few feeder roots. These also grow in the east/west direction.

According to Bray (1), nutrients such as phosphorus, potassium, calcium, etc., are relatively immobile and their availability to plants is limited by the nature of the plant, particularly the density and extensiveness of the rooting system. The same author (1) also states that the roots must go out and forage for the immobile nutrients, continually sending out new roots as the older ones exhaust the effective feeding zone. The general practice of repeated close machine cultivation between the beet rows will cut off feeder roots when rows are planted in north/south direction. The characteristic of the feeder roots to grow in an east/west direction could be used to advantage by placing fertilizer in line with feeder root growths. Therefore, a preliminary test was conducted at two locations to study the effect of various fertilizer placements on yield and quality of the crop. The field at location "A" was a somewhat poorly drained, heavy clay soil. The field at location "B" was a fine sandy clay loam. Both fields were of average fertility and had been summer-fallowed in the previous year.

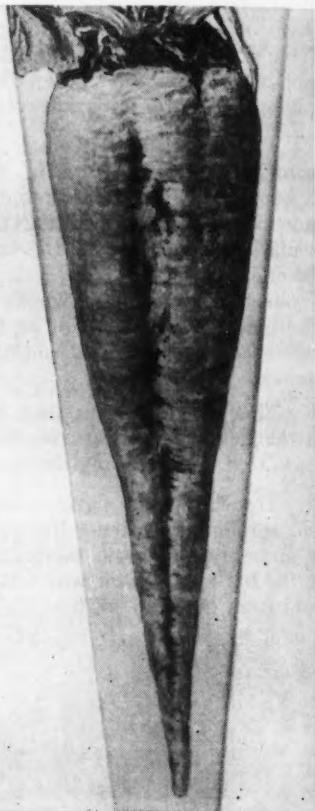


FIGURE 1. Sketch of sugar beet, illustrating appearance of typical crease.

TABLE I.—THE AVERAGE EFFECT OF FERTILIZER PLACEMENT ON FOLIAGE AND ROOT WEIGHTS OF SUGAR BEETS AT TWO LOCATIONS

Planting direction and time of harvest	Foliage weight: Tons/acre				Root weight: Tons/acre			
	(a)	(b)	(c)	L.S.D.	(a)	(b)	(c)	L.S.D.
East/West, pre-harvest	9.77	10.05	10.78	N.S.	3.90	4.01	4.35	N.S.
North/South pre-harvest	8.60	9.10	10.80*	1.02	3.93	3.73	4.27	N.S.
East/West final harvest					12.80	13.80*	14.23*	0.63
North/South final harvest					13.15	13.48	14.90*	0.78

(a) Check plots

(b) 60 lb. 11-48-0 side-dressed

(c) Same as in (b), plus 60 lb. 11-48-0 deep placed

\* Significant increase over check at the 5% level

At each location two identical experimental plots were laid out, side by side. In one experimental plot the beets were planted with rows in an east/west direction. In the other the rows were planted in a north/south direction. The designs were randomized complete blocks with six replications of three treatments each. Each treatment consisted of eight rows, or two sets of four rows, to provide a pre-harvest and the final harvest. At the pre-harvest, made on August 1, yields of roots and foliage were taken. At the final harvest, on October 16, yield and sucrose content of the roots were determined. The treatments consisted of: (a) untreated (check) plots; (b) 60 lb. of ammonium-phosphate (11-48-0) applied at seeding-time with a precision planter, in bands approximately  $1\frac{1}{2}$  inches to the side of the beet row and at a depth of approximately 2 inches; (c) the same treatment as in (b), plus 60 lb. of 11-48-0, applied with a specially constructed applicator 2 days later at a depth of approximately  $5\frac{1}{2}$  inches in bands along the beet rows. The beets were planted about the middle of May and all operations of the two experiments at each station were carried out simultaneously. The individual plots consisted of four rows 20 inches apart and 60 feet long. The centre two rows were trimmed to 50 feet in length and harvested for yield data.

The effect of the various treatments for both planting directions was consistent at both locations. The data were averaged and are presented in Table I.

At the time of the pre-harvest sampling, the most important factor was foliage development. Major root development occurs later in the growing season.

#### *Pre-harvest*

In the north/south direction, the 60 lb. application of 11-48-0 did not significantly increase the foliage development over the check. However, the application of 120 lb. of 11-48-0, half at seeding and half as deep placement, increased the foliage weight significantly over both the check and the 60 lb. rate. In the east/west planting direction no significant response was obtained.

### *Final Harvest*

- (a) *East/west planting direction.* A significant increase over the check was obtained by the application of 60 lb. of 11-48-0 applied at planting. An additional application of 60 lb. of 11-48-0 as deep placement did not increase the yield significantly over the single application.
- (b) *North/south planting direction.* In contrast to the east/west direction, the application of 60 lb. of 11-48-0 at planting time did not increase the yield significantly over the check. However, the additional application of 60 lb. of 11-48-0 as a deep placement did increase the yield significantly over the single application.

There was no significant difference in sucrose content from the various treatments. The gross sugar return, therefore, followed the root yield pattern.

The general fertilizer recommendations for sugar beets grown in the Red River Valley in Manitoba is 60 lb. of 11-48-0 per acre in bands at seed-ing-time, at a depth of approximately 2 inches. In the past the response to this treatment has been rather erratic and higher rates failed consistently to give additional increases.

The differential response obtained in this experiment is in agreement with what should be expected with east/west orientation of feeder roots. When rows are planted in north/south direction close cultivation tends to cut off the feeder roots on the sides of the beets near the surface and thus encourages root development at a lower level. This type of development is evidenced by the response to deep placement of fertilizer. Conversely, when rows are planted in an east/west direction, the feeder roots tend to develop in the row, undisturbed by cultivation, thus taking advantage of the shallow placed fertilizer. Since sufficient nutrients were obtained at this level no further increase was obtained from the deeper placed fertilizer.

Experiments are under way to substantiate these preliminary findings which appear to explain, at least in part, the erratic response obtained from applications of 60 lb. of 11-48-0 per acre to sugar beets in Manitoba.

### REFERENCE

1. Bray, Roger H. "Correlation of soil tests with crop response to added fertilizers and with fertilizer requirement". In *Diagnostic techniques for soils and crops*. American Potash Institute, Washington, D.C. 1948.

—K. SCHREIBER,  
The Manitoba Sugar Company Limited,  
Fort Garry, Manitoba

May 24, 1957



**Erratum:**

It is regretted that, through a printers' error, the name of **R. L. Halstead**, Chemistry Division, Science Service, Canada Department of Agriculture, Ottawa, was not shown, as co-author with H. B. Heeney, of the paper published on page 347 of the October, 1957, issue (Volume 37, No. 4), entitled "*Further studies of the relationship of soil phosphorous tests to the phosphatic fertilizer requirement of the tomato*". The joint authorship was correctly shown on the "Contents" page of the above issue.



